UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

GRADUATE EDUCATION
(301) 295-3913
FAX: (301) 295-6772

# APPROVAL SHEET

Title of Dissertation: "Identification of CS-derived Compounds Formed During Heat Dispersion of CS Riot Control Agent and the Temperature Ranges Associated with their Formation"

Name of Candidate: CPT Timothy Kluchinsky
Doctor of Public Health
8 June 2001

Dissertation and Abstract Approved:

David Cruess, Ph.D. — 25 June 2001
Department of Preventive Medicine & Biometrics
Committee Chairperson

LCDR Philip Smith, USN — 8 JUNE 2001
Department of Preventive Medicine & Biometrics
Committee Member

Arthur Lee, Ph.D. — 8 June 01
Department of Preventive Medicine & Biometrics
Committee Member

CAPT Richard Thomas, USN — 8 Jun 01
Department of Preventive Medicine & Biometrics
Committee Member

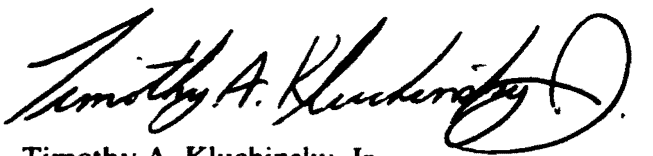
COL Clifford Cloonen, USA — 8 June 01
Department of Military& Emergency Medicine
Committee Member

Robert Fitz, MPH, MSPH — 8 June 01
Department of Preventive Medicine & Biometrics
Committee Member

The author hereby certifies that the use of any copyrighted material in the dissertation manuscript entitled:

**"IDENTIFICATION OF CS-DERIVED COMPOUNDS FORMED DURING HEAT DISPERSION OF CS RIOT CONTROL AGENT AND THE TEMPERATURE RANGES ASSOCIATED WITH THEIR FORMATION"**

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.



Timothy A. Kluchinsky, Jr.
CPT, MS, U.S. Army
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences

# ABSTRACT

## "IDENTIFICATION OF CS-DERIVED COMPOUNDS FORMED DURING HEAT DISPERSION OF CS RIOT CONTROL AGENT AND THE TEMPERATURE RANGES ASSOCIATED WITH THEIR FORMATION"

by

Timothy A. Kluchinsky, Jr, Doctor of Public Health

Uniformed Services University of the Health Sciences, 2001

Dissertation Advisor: Philip A. Smith, Ph.D.
Department: Preventive Medicine and Biometrics
Division: Environmental and Occupational Health

**Background:** Military, correctional officer, and law enforcement personnel, are routinely exposed to 2-chlorobenzylidenemalononitrile (CS) during combat training, chemical protective mask confidence training, and riot control operations training. The public may be involuntarily exposed during rioting or civil disturbances. The potential exists for CS to form new compounds when dispersed by means of oxidizer-supported combustion of a chemical fuel from inside a canister.

**Objectives:** Research objectives were to identify CS-derived compounds that may be relevant to the preservation of health among those exposed, and to explore the effect of temperature on their formation. In addition to the need to identify these compounds and to understand their toxicity, knowledge of the temperature ranges associated with their

formation is also important. If CS-derived compounds produced during heat-dispersion of CS canisters prove to be a public health hazard, alternative dispersion methods may be warranted.

**Specific Aims:** Research aims were to report on the objectives discussed above, publish the analytical methods and instrumental techniques necessary to characterize CS-derived compounds, and explore conditions leading to their formation.

**Study Design:** Air samples collected during heat-dispersion of CS were analyzed using open tubular gas chromatography coupled to mass spectrometry and compared to known standards using spectral interpretation techniques guided by spectral library comparison. Additional analytical methods were employed to explore formation of select inorganic analytes. A tube furnace was used to explore the effect of temperature on formation of CS-derived compounds.

**Results:** Compounds observed in air sample filter extracts, in addition to CS included 2-chlorobenzaldehyde, 2-chlorobenzonitrile, quinoline, 2-chlorobenzylcyanide, 1,2-dicyanobenzene, 3-(2-chlorophenyl)propynenitrile, *cis* and *trans* isomers of 2-chlorocinnamonitrile, 2,2-dicyano-3-(2-chlorophenyl)oxirane, 2-chlorohydrocinnamonitrile, benzylidenemalononitrile, *cis* and *trans* isomers of 2-cyanocinnamonitrile, 2-chlorobenzylmalononitrile, 3-quinoline carbonitrile, 3-isoquinoline carbonitrile, 4-chlorobenzylidenemalononitrile, hydrogen cyanide, and hydrogen chloride. Experimentation using a tube furnace showed that formation of most of the CS-derived compounds observed was temperature dependent. The results of this research support the need for future evaluation of exposure to potentially harmful CS-derived compounds.

Control of such exposures through implementation of appropriate personal protective equipment or alternative delivery methods may be warranted.

# "IDENTIFICATION OF CS-DERIVED COMPOUNDS FORMED DURING HEAT DISPERSION OF CS RIOT CONTROL AGENT AND THE TEMPERATURE RANGES ASSOCIATED WITH THEIR FORMATION"

by

Captain Timothy A. Kluchinsky, Jr.
Environmental Science Officer
United States Army

A dissertation submitted to the Faculty of the

Department of Preventive Medicine and Biometrics,

Uniformed Services University of the Health Sciences

in partial fulfillment of the requirements for the degree

of

DOCTOR OF PUBLIC HEALTH, 2001

## DEDICATION

I dedicate this doctoral dissertation to my lovely wife, Aunika. This work would not have been possible, and the opportunity to become a doctor would not have been grasped wholeheartedly, if it were not for her commitment to ensuring me that the goals that I had set for myself early in life, had become family goals. It has certainly been a total TEAM effort. And I love her very much for that.

-Tim

## ACKNOWLEDGMENTS

I am grateful to the coauthors of the manuscripts included in this dissertation, the Uniformed Services University of the Health Sciences, the United States Army Center for Health Promotion and Preventive Medicine, the Henry Jackson Foundation, and Brigham Young University for the assistance, guidance, and support provided in making this study possible. Their commitment to preservation of military and public health has been instrumental in the development of this dissertation.

I would like to thank all the members of my committee and their respective academic divisions for their time, patience, and confidence: David F. Cruess, Ph.D, Professor & Deputy Chairperson, Biometrics, Department of Preventive Medicine and Biometrics (Committee Chairperson); LCDR Philip A. Smith, M.P.H., Ph.D., Assistant Professor, Division of Environmental and Occupational Health, Department of Preventive Medicine and Biometrics (Major Dissertation Advisor); LTC(ret) Arthur P. Lee, Ph.D., Assistant Professor, Division of Environmental and Occupational Health, Department of Preventive Medicine and Biometrics (Academic Advisor); CAPT Richard J. Thomas, M.D., M.P.H., Assistant Professor and Director, Division of Epidemiology and Biostatistics, Department of Preventive Medicine and Biometrics; COL Clifford C. Cloonan, M.D., Vice Chairperson, Department of Military Emergency Medicine, Emergency Medicine Consultant to the Office of the Surgeon General; and COL(ret) Robert Fitz, M.P.H., M.S.P.H., Assistant Professor, Division of Environmental and Occupational Health, Department of Preventive Medicine and Biometrics.

I am especially thankful to my major advisor LCDR Philip A. Smith who accepted the remarkable challenge of developing a young Army Captain into an

environmental/public health scientist possessing the skills necessary to scientifically evaluate and address environmental and public health concerns.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# CHAPTER 1

# INTRODUCTION

## *HISTORY OF THE USE OF " GASES"*

The earliest case involving the use of "gas" (referring to the dispersal of irritants as gases, vapors, or aerosols) as a weapon may have occurred during the Athenian-Spartan War of 428 B.C. when the Spartans set a mixture of wood, pitch, and sulfur afire at the base of the wall of the Athenian city Plataea, in an attempt to incapacitate the resisting Athenian forces [1] [2]. Plutarch, a Greek writer during the early Roman period, recorded five hundred years later that the Romans were successful in using irritants as a method of cave denial in Spain during the first century A.D. [3].

Riot control agents are commonly referred to as irritants, harassing agents, or "tear gases" (again, not necessarily gas phase materials). There are three types of riot control agents: lacrimators, which cause mainly lacrimation and eye irritation; sternutators, which cause mainly sneezing and irritation of the upper respiratory tract; and vomiting agents, which cause vomiting in addition to some of the effects caused by lacrimators and sternutators. Chloropicrin (trichloronitromethane, PS), synthesized from picric acid (2,4,6-trinitrophenol) and calcium hypochlorite in 1848, was the first of the more than 30 known tear gases to eventually be developed. It was used extensively as a sternutator during World War I; however, its use as a riot control agent was limited due to its high toxicity characteristics [4]. By 1899, concern over the use of poison gases, including irritants, grew to a point where the major world powers saw a need to come to a common agreement regarding their use. While meeting at The Hague in 1899, twenty-seven nations representing the major world powers agreed to a declaration "to abstain

from the use of projectiles the object of which is the diffusion of asphyxiating or deleterious gases". Germany, unlike Great Britain and the United States, was among the nations agreeing to the declaration [5].

Irritants were deployed during World War I by the French, Germans, British, and United States. Germany, in keeping with the objectives of the 1899 Hague Convention, produced artillery shells designed to deliver both an explosive charge and an irritating agent. They soon abandoned this use of irritants, largely due to their inability to vaporize in cold weather, in favor of chlorine gas. Germany's use of chlorine at Ypres, Belgium on 22 April 1915 is commonly referred to as the beginning of chemical warfare on the modern battlefield, even though the French had used the irritant ethyl bromoacetate eight months earlier. When the war ended in November 1918, the United States had suffered 258,338 casualties. 70,552 (31.4%) of those casualties were caused by chemical warfare. Some of the other irritants deployed by forces during the war consisted of ethyl bromoacetate, chloroacetone, xylyl bromide, benzyl bromide, and bromobenzylcyanide [4]. The eye irritant alpha-chloroacetophenone (CN), discovered by the German chemist Graebe in 1869 [6], was produced by the United States during World War I. However, large-scale production facilities were not completed prior to the end of the war. As a result, CN's primary use was as a riot control agent between World War I and World War II by law enforcement personnel.

The Geneva Convention Gas Protocol of 1925 was drafted as a consequence of the use of chemical and riot control agents during World War I [7]. The protocol defined the use of riot control agents as equivalent to the use of chemical warfare agents. The United States refused to recognize the gas protocol because it believed that chemical

warfare agents did not include riot control agents. The United States held firm in their objections to the protocol for fifty years. It was not until the conclusion of the Vietnam War, which involved heavy use of 2-chlorobenzylidenemalononitrile (CS, Figure 1-1) by the United States for tunnel denial, that President Gerald R. Ford acknowledged the 1925 Gas Protocol by signing Executive Order 11,850 [8]. The executive order renounced the use of riot control agents in armed conflict except when used in rescue operations, rear echelon operations, anti-civilian screening operations, and riot control operations in areas under direct United States military control. The current policy regarding use of riot control agents during wartime requires prior presidential approval regardless of whether it is first use or retaliatory use [9].

*BACKGROUND AND SIGNIFICANCE*

In 1928, Corson and Stoughton first synthesized several benzylidene malononitriles by condensing aromatic aldehydes with malononitrile [10]. One of these compounds, 2-chlorobenzylidenemalononitrile (the code name "CS" is derived from the chemists' initials), was shown to have an intense sneeze and skin irritant effect, and they noted that exposure to it caused the face to smart. This outcome, which can be minimized by using a protective (gas) mask, is intensified if water is used to rinse oneself of the compound. The characteristics of CS made it a noteworthy candidate for widespread adoption as an incapacitant. However, it wasn't readily accepted as one until well after World War II, when it was learned that the effect of CN was more toxic and less potent than that of CS [6, 9]. Symptoms for CS exposure consist of severe burning

of the eyes, nose and throat. Exposure to CS causes individuals to close their eyes and hold their breath, immediately rendering them incapacitated.

Most of the forces involved in World War II were capable of producing CN prior to the end of the war. International concerns regarding the effectiveness of CN peaked in 1958 when the British noticed that rioters and enemy soldiers of Cyprus were able to proceed with normal operations while in a CN environment by practicing eye rinsing and breathing through bleach or alcohol-soaked cloths. These events, adding to the compound's chemical instability, resulted in the need to produce a more effective agent for riot control. During the 1950's, the Chemical Defence Experimental Establishment of Porton Down, England re-examined the World War I agent bromobenzylcyanide (CA, Figure 1-2) as an alternative to CN. By adding a carbon and a chlorine atom, replacing the bromine atom with another cyano group, and creating an alkene at the alpha-beta carbons, they arrived at the structure for CS, the compound originally discovered by Corson and Stoughton in 1928. Synthesis mechanisms were given to the North Atlantic Treaty Organization (NATO) members and in fall 1959, CS became the standard NATO military incapacitant. By the early 1960's, CS had replaced CN in many military and law enforcement storage facilities [9].

Like CN, CS rapidly loses its effectiveness under normal environmental conditions making it an ideal temporary incapacitant. The United States Department of Defense created two hydrophobic variations in addition to the non-persistent form of CS, and named them CS1 and CS2. CS1 is a micronized powder containing 5 % hydrophobic silica aerogel similar to common desicants. CS2 is a siliconized microencapsulated form

of CS1 with shelf-life, persistence, resistance to degradation, and ability to float on water providing a means of restricting the use of water for military operations [11].

Adding to its extensive use in Vietnam, CS has been effectively used in the United States as a riot control agent for demonstrations and prison riots, as well as in military and law enforcement training. In 1993, the Branch Davidian Complex, located in Waco, Texas was raided by the Bureau of Alcohol, Tobacco, and Firearms (ATF) in an effort to serve arrest and search warrants as part of an investigation into illegal possession of firearms and explosives. Law enforcement officers used CS in an attempt to remove the members of the congregation from the complex [12]. Most recently, CS was deployed in an attempt to gain control of an estimated 40,000 demonstrators in Seattle, Washington where the 2000 World Trade Organization Meeting hosted 134 member nations [13].

CS is commonly used as a riot control agent and chemical warfare agent simulant for training. Employees of law enforcement and military organizations are routinely exposed to heated CS during training. Heat assists in the CS dispersion process by vaporizing the CS, which then condenses to form an aerosol. Heat-dispersion of CS has the potential to form CS-derived compounds that have not been evaluated for the hazards they may pose not only to law enforcement, correctional, and military personnel, but to the general public as well.

Current Occupational Safety and Health Administration (OSHA)/National Institute of Occupational Safety and Health (NIOSH) analytical methods for CS require two-stage air sampling using a polytetraflouroethylene (PTFE) filter and Tenax$^{TM}$ sorbent tube, extraction using 20% methylene chloride in hexane, and analysis by high

performance liquid chromatography (HPLC) using an ultraviolet detector at a wavelength of 305 nanometers [14]. This analytical method is not well-suited for electron impact mass spectral analysis of CS-derived compounds formed during heat-dispersion of CS, because the liquid mobile phase flow is two to three orders of magnitude greater than that which mass spectrometer (MS) vacuum systems are generally capable of removing for electron impact ionization [15].

Our research group's interest in the safety of CS being used for military training stems from the occurrence of a 1996 field exposure incident that appears to be associated with the hospitalization of nine otherwise healthy Marines for transient pulmonary syndrome of cough, shortness of breath, hemoptysis, and hypoxia. Four of these Marines were hospitalized in an intensive care unit due to hypoxia, although they did not require ventilator-assisted care [16]. All of the hospitalized Marines were subsequently "dropped from rolls" of the amphibious reconnaissance school they were attending, and returned to their units. Hence, tarnished careers may have resulted from individuals' apparent unexplained adverse effects from exposure to CS and CS-derived compounds.

*RESEARCH OBJECTIVES*

Research objectives were to (1) identify CS-derived compounds formed during heat-dispersion of CS, and (2) explore the effect of temperature on their formation. If necessary, minimization of exposure may be accomplished through implementation of appropriate personal protective equipment requirements and use of alternative delivery methods. In addition to the need to identify these CS-derived compounds and to understand their toxicity, knowledge of the temperature ranges associated with their

formation is also important. If CS-derived compounds produced during heat-dispersion of CS canisters prove to be a public health hazard, alternative dispersion methods may be warranted.

Among the compounds that were suspected of being formed during heat-dispersion of CS were CS epoxide, hydrogen cyanide (HCN), hydrogen chloride (HCl), and several additional compounds potentially produced through free radical intermediates [17]. CS epoxide is a strong vesicant capable of producing third-degree burns [18]. Exposure to sustained sublethal concentrations of HCN is thought to adversely affect the brain and nervous system [19]. HCl is mildly toxic by inhalation and a very powerful eye, skin, and mucous membrane irritant [20]. Free radicals are molecular fragments possessing unpaired electrons that have been produced through homolysis of covalent bonds [21]. Free radical chemical species are potentially highly reactive and may pose exposure health risks.

*CHARACTERISTICS OF CS*

CS is a white, crystalline solid having a pepper-like odor. Additional characteristics are a molecular weight of 188.6; boiling point of 310-315°C; melting point of 95-96°C; and vapor pressure of $3 \times 10^{-5}$ mmHg. It is hardly soluble in water but very soluble at 25°C in organic solvents such as methylene chloride, acetone, ethyl acetate, benzene, and dioxane [22].

The chemical structure of CS promotes an electron deficiency at the alpha-beta double bond, resulting in its toxicity and strong incapacitating ability. Pi bond resonance of the benzene ring contributes to the compound's stability. A study by Leadbeater *et al.*

concluded that when the CS double bond is hydrogenated to form *ortho*-chlorobenzylmalononitrile ($CSH_2$), the LD50 (lethal dose for 50% of the test subjects) in mice increases from 47.7 (95% CI, 42.9-55.8) to 78.4 mg/kg (95% CI, 47.2-127.0). Additionally, the results of the guinea pig blepharospasm irritancy test for $CSH_2$ were fifteen times less than that of CS, indicating a decrease in irritancy [23].

*CLINICAL PRESENTATION OF CS EXPOSURE*

CS exposure to the eyes results in a burning sensation, transient conjunctivitis for up to 30 minutes, blepharospasms, eyelid erythema, photophobia, and intense lachrymation [24].

Exposure to the respiratory tract results in a painful, burning sensation beginning at the throat and continuing down the trachea and bronchi. Burning also occurs in the nose, accompanied by rhinorrhoea, epistaxis, and erythema of the nasal mucous membranes. Nausea, headache, sneezing, diarrhea, choking, coughing, fatigue, and sometimes vomiting have been reported after exposure [24].

A burning sensation occurs upon exposure to the skin. This sensation is amplified when the area is moistened and may recur while washing the area hours later. Chronic skin exposure to large quantities of CS can result in erythema and blistering.

CS particle size affects the clinical result. Heat-dispersion mainly results in the formation of a fine aerosol containing particles with a mass median diameter of 0.5-2.0 microns. Small airborne particles less than 1 micron in diameter, predominately affect the respiratory tract. Larger particles have a more pronounced effect on the eyes than the respiratory tract [25].

*TOXICITY OF CS*

Human exposure data indicated that a 2-minute exposure to air concentrations in the range of 2-10 mg/m$^3$ was considered "intolerable" by 6 of 15 individuals exposed [26]. Soon afterwards, the median incapacitating concentration range was determined to be from 12 to 20 mg/m$^3$ for a 20 second exposure [27]. In a study performed to identify the outcome of a low level exposure, it was noted that 3 of 4 volunteers exposed to 1.5 mg/m$^3$ for 90 minutes developed headaches. The same study noted that human volunteers found concentrations greater than 10 mg/m$^3$ to be extremely irritating and intolerable for more than 30 seconds because of burning and pain in the eyes and chest [28]. A later study also showed that exposures above 14 mg/m$^3$ for 1 hour produced extreme irritation, erythema, and vesication to the skin of volunteers [29]. One study evaluated the human dermal exposure range of 2 mg to 30 mg for dry and moistened CS applied to a 4 cm in diameter, circular area of the forearm of healthy volunteers. It concluded that only at 20 mg for dry and 10 mg for moistened CS, was there a faint erythema and irritation lasting up to 30 minutes following exposure [30].

CS produces lachrymation, eye pain, and discomfort in the upper respiratory tract and chest when dispersed as a smoke consisting mainly of 1-micron diameter particles. Recovery may occur as soon as a few minutes after being placed in a fresh air environment. Several authors have experimented with CS using human respiratory function tests [31, 32] and concluded that their studies did not reveal any effect of CS on lung function, pulmonary gas transfer, or alveolar volume using the single breath carbon monoxide method after a one hour exposure to mean concentrations ranging from 0.62 mg/m$^3$ to 2.00 mg/m$^3$.

In 1963, Punte *et al.* showed that CS, in the presence of hypochlorite-containing bleach, produced 2,2-dicyano-3-(2-chlorophenyl)oxirane (CS epoxide). Controlled human exposures to a CS and bleach mixture resulted in 8 of 11 subjects receiving second-to-third degree burns [28], probably due to CS epoxide exposure. Four years later, the United States Army patented a process for making epoxides from ethylenic compounds with electron-withdrawing groups. One of the epoxides noted in the patent was CS epoxide, observed to possess strong vesicant ability and usefulness as a chemical warfare agent [18]. A literature search using the CS epoxide Chemical Abstracts Service (CAS) number (3515-08-4) and other naming strategies [3-(2-chlorophenyl)-2,2-oxiranedicarbonitrile ($9^{th}$ comprehensive index); 3-(o-chlorophenyl)-2,2-oxiranedicarbonitrile ($8^{th}$ comprehensive index)] using the CAS database and the Chemical and Biological Defense Information Analysis Center (CIBIAC) resulted in only five citations containing any mention of CS epoxide [18, 19, 33-35].

It appears that CS epoxide may form when a strong oxidizer is introduced into a CS system. Punte *et al.* did this in the previously mentioned human exposure study using hypochlorite mixed with CS [28]. In analyses done by our research group, we have observed that when glass wool from a Tenax™ sorbent tube is introduced into a vial containing CS extract dissolved in toluene, CS epoxide forms immediately and quantitatively increases for up to at least seven days. The CS epoxide probably forms due to the availability of active sites on the glass wool and the availability of oxygen in the vial system.

*GAS CHROMATOGRAPHY / MASS SPECTROMETRY*

Samples collected during this research were analyzed using open tubular gas chromatography with mass spectrometric detection. Gas chromatography (GC) is a method for separating sample components by using unequal component distributions in two phases: a mobile gas and a stationary phase [36]. The mobile gas phase, containing the sample, travels through a column enroute to separation based on polarity or boiling point, depending on the type of column. A retention time is characteristic for a given compound using a specific type of column stationary phase, mobile phase flow rate, and column oven temperature program. The GC may be coupled with any of numerous detectors to include a flame ionization detector, electron capture detector, or mass spectrometer.

Electron impact mass spectrometry (MS), the detector of choice for this research following GC, is a powerful analytical tool used to quantify known compounds and identify/quantify unknown compounds by producing mass spectra of those compounds. In GC/MS, molecules enter the MS high vacuum region as they elute from the GC column. The molecules are ionized and fragmented upon entering the ion source. The positively charged fragments are subsequently separated according to mass. A signal is produced that is proportional to the number of ions present for each mass. A summary of the mass of the ions and their abundance may be plotted. This procedure facilitated the identification of CS thermal degradation products present in our samples.

The analysis of CS using gas liquid chromatography was first documented in 1971 [37] and later with methods using ultraviolet, infrared, fluorescence, and proton nuclear magnetic resonance (NMR) [38]. MS is widely used for the identification of

unknown compounds. Several authors have analyzed CS using GC followed by MS [38-41]. The mass spectra of CS, a few of its cyano-containing derivatives, and *ortho*-chlorobenzaldehyde were obtained by Wils and Hulst [33, 34].

The OSHA/NIOSH methods for CS analysis described earlier require HPLC and ultraviolet detection. The HPLC analytical method is not optimal for the analysis for CS and the investigation of its thermal degradation products as discussed previously. However, analysis is readily available using GC and electron impact MS--our method of choice.

*HEALTH RISK ASSESSMENT*

Identification of the compounds of concern, followed by toxicity assessment, exposure assessment, and risk characterization are the four steps of a baseline human health risk assessment (HRA) [42]. The HRA is a United States Environmental Protection Agency (USEPA) method of developing health risk information at Superfund Sites and is designed to estimate the increased cancer and non-cancer hazard risks posed due to exposure to identified compounds of concern. *The Human Health Evaluation Manual (Volume I),* which contains the USEPA's guidance for conducting an HRA, replaced a previous USEPA guidance document titled *The Superfund Public Health Evaluation Manual* (October 1986).

Identification of the compounds of concern is the first step of the HRA and is accomplished through field sampling and laboratory analyses for potential contaminants. The second step of an HRA is a toxicity assessment that considers: (1) the adverse health effects associated with exposure to compounds of concern; (2) the relationship between

these adverse effects and the magnitude of exposure; and (3) the related uncertainties associated with the compounds' carcinogenicity in humans. An exposure assessment is the third step of the HRA and is designed to estimate the actual and/or potential magnitude, frequency, duration, and pathway of exposure to the compounds of concern. The last step is a risk characterization that estimates cancer risks and noncancer hazard quotients based on the results of the first three steps of the HRA.

*MANUSCRIPTS*

The first manuscript titled "*Identification of CS-derived Compounds formed during Heat-dispersion of CS Riot Control Agent*" may be considered as part of the initial step of a comprehensive HRA of CS and CS-derived compounds produced by heat-dispersion. It identifies organic CS-derived compounds produced from CS dispersed from canisters. Most of these compounds have not been evaluated for their toxicity. Previous studies to assess the health risks associated with exposure to heat-dispersed CS evaluated CS independently; thereby, not including the health risks associated with exposure to the potentially harmful CS-derived compounds produced.

The second manuscript titled "*Liberation of Hydrogen Cyanide and Hydrogen Chloride during High Temperature Dispersion of CS Riot Control Agent,*" provides data that add HCN and HCl to the list of compounds of concern identified in the first manuscript by confirming the presence of these gas phase salt type contaminants during heat-dispersion of CS. Of particular importance was the apparent potential to produce HCN as an air contaminant.

A third manuscript titled *"Formation of 2-chlorobenzylidenemalononitrile (CS Riot Control Agent) Thermal Degradation Products at Elevated Temperature"* discusses which compounds of concern were produced in an inert atmosphere at tube furnace temperatures of 300, 500, 700, and 900°C while in a laboratory setting. If CS-derived compounds produced during heat-dispersion of CS canisters prove to be a public health hazard, alternative dispersion methods, such as a volatilization at lower temperatures may be warranted.

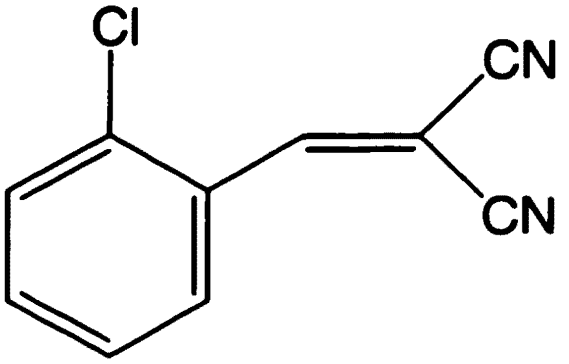



**Figure 1-1.** 2-chlorobenzylidenemalononitrile (CS)

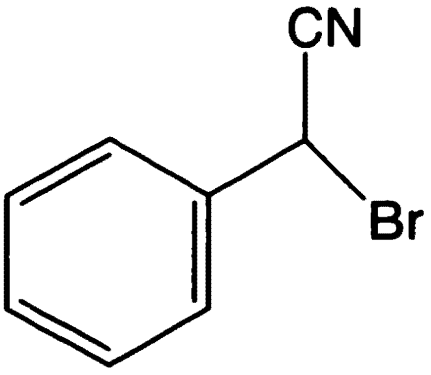



**Figure 1-2.** Bromobenzylcyanide (CA)

# CHAPTER 2

## Identification of CS-derived Compounds Formed during Heat-dispersion of CS Riot Control Agent

[1]Timothy A. Kluchinsky, Jr., [2]Paul B. Savage, [3]Michael V. Sheely, [1]Richard J. Thomas, *[1]Philip A. Smith [1]Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, Bethesda MD, [2]Brigham Young University, Department of Chemistry and Biochemistry, Provo UT, [3]United States Army Center for Health Promotion and Preventive Medicine, Aberdeen MD, *Corresponding author

**Abstract:** High temperatures are frequently used to disperse 2-chlorobenzylidene-malononitrile (CS) riot control agent. We examined airborne CS degradation products heat-dispersed together with CS from canisters of a type used by law enforcement personnel for crowd and riot control. Air contaminants derived from CS were trapped using a polytetraflouroethylene filter. Analysis was by open tubular gas chromatography coupled to mass spectrometry. Compounds observed in addition to CS included 2-chlorobenzaldehyde, 2-chlorobenzonitrile, quinoline, 2-chlorobenzylcyanide, 1,2-dicyanobenzene, 3-(2-chlorophenyl)propynenitrile, *cis* and *trans* isomers of 2-chlorocinnamonitrile, 2,2-dicyano-3-(2-chlorophenyl)oxirane, 2-chlorohydrocinnamo-nitrile, benzylidenemalononitrile, *cis* and *trans* isomers of 2-cyanocinnamonitrile, 2-chlorobenzylmalononitrile, 3-quinoline carbonitrile, 3-isoquinoline carbonitrile, and 4-chlorobenzylidenemalononitrile

**Key words:** CS riot control agent; degradation products; open tubular gas chromatography; mass spectrometry

This manuscript has been completed in partial fulfillment of the degree of Doctor of Public Health, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the United States Department of Defense or the Uniformed Services University of the Health Sciences.



## INTRODUCTION

2-chlorobenzylidenemalononitrile (CS) was first synthesized by Corson and Stoughton in 1928 [1]. During the late 1950's and early 1960's, military organizations and civilian law enforcement agencies accepted CS as a replacement for the riot control agent *alpha*-chloroacetophenone (CN), which has been described as "more toxic and less effective than CS" [2].

Early efforts to analyze CS present in mixtures by gas chromatography (GC) used packed columns (mostly glass) [3-7]. The modern use of open tubular gas chromatography (OTGC) columns with bonded stationary phases has led to great improvements in chromatographic efficiency, complex mixture analysis, and coupling of GC separation methods to mass spectrometry (MS). OTGC is the separation method of choice for detection and identification of analytes using electron impact (EI) ionization mass spectrometry. Although Wils and Hulst [8] used GC with a capillary column and EI-MS to identify "impurities, degradation products and reaction products with solvents" related to CS, OTGC methods have mostly been used to analyze CS, with little attention paid to degradation products or other analytes present with the primary CS analyte [9-12].

Smith *et al.* used OTGC/MS analysis to identify several of the CS degradation products observed as air contaminants from heat dispersion of CS [13]. The work reported here follows synthesis of standards required to complete the 70 eV EI mass spectral identification of many of the organic analytes observed in the work of Smith *et al.*

## EXPERIMENTAL

*Materials.* Continuous discharge, Type 3, CS canisters were purchased from Armor Holdings Inc. (Jacksonville, FL). Dichloromethane solvent was obtained from AlliedSignal Inc. (pesticide grade, Muskegon, MI). Analytical standards were purchased from Aldrich (Milwaukee, WI) for CS (97%), 2-chlorobenzaldehyde (99%), 2-chlorobenzonitrile (98%), quinoline (98%), 2-chlorobenzylcyanide (98%), 1,2-dicyanobenzene (98%), 2-chlorohydrocinnamonitrile (99%), benzylidenemalonontrile (98%), 3-quinoline carbonitrile (99%), 3-isoquinoline carbonitrile (99%) and 4-chlorobenzylidenemalononitrile (unknown purity).

Standards for 2-cyanocinnamonitrile (*cis* and *trans*), 2-chlorocinnamonitrile (*cis* and *trans*), 2-chlorobenzylmalononitrile (dihydro CS), 3-(2-chlorophenyl)propynenitrile, and 2,2-dicyano-3-(2-chlorophenyl)oxirane (CS epoxide), were not available from commercial sources; consequently, it was necessary to synthesize these standards. The *cis* and *trans* isomers of 2-cyanocinnamonitrile were prepared by treating 2-cyanobenzaldehyde (Aldrich, 98%) with (triphenylphosphoranylidene)acetonitrile (Aldrich 97%) in ethyl acetate. A similar approach was followed to produce *cis* and *trans* isomers of 2-chlorocinnamonitrile with 2-chlorobenzaldehyde taking the place of 2-cyanobenzaldehyde in this synthesis. The *cis* and *trans* isomers of 3-cyanocinnamonitrile

and 4-cyanocinnamonitrile were synthesized in order to rule out the presence of similar isomers having the cyano group at the *meta* and *para* positions on the benzene ring. Preparation of these compounds was accomplished by adding either 3-cyanobenzaldehyde (Aldrich, 98%) or 4-cyanobenzaldehyde (Aldrich, 95%) respectively to (triphenylphosphoranylidene)acetonitrile as described above. The *cis* and *trans* isomers of 2-cyanocinnamonitrile and 2-chlorocinnamonitrile were separated by flash column chromatography (silica gel as stationary phase, ethyl acetate-hexanes as eluent). Isomers were identified by characteristic vinylic $^{1}H$ NMR coupling constants. 2-Chlorobenzylmalononitrile was prepared by treating 2-chlorobenzyl bromide with malononitrile and piperidine in tetrahydrofuran (THF). The 3-(2-chlorophenyl)propynenitrile standard was prepared by reaction of a mixture of *cis* and *trans*-2-chlorocinnamonitrile with bromine followed by two sequential dehydrobromination reactions. The first, yielding a mixture of *E* and *Z* 3-(2-chlorophenyl)propenitrile, was achieved using triethylamine. The second dehydrobromination, yielding the corresponding alkyne, involved treatment of the *E* and *Z* mixture with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in THF. Only one of the isomers, presumably *Z*, gave the alkyne; the other alkene remained unchanged. Separation of the alkyne from the unreacted alkene provided the necessary standard. CS epoxide was prepared using the methodology of Harrison and Inch [14]. For all standards synthesized, structures were confirmed using $^{1}H$ NMR, $^{13}C$ NMR, and high resolution-fast atom bombardment MS in addition to the GC/MS methods reported here.

*Air Sampling.* Two CS riot and crowd control canisters were placed in the center of an enclosed 240 $m^3$ chamber used by law enforcement agencies for training with riot

control agents. The CS and degradation products sampled were thermally dispersed from the canisters through the normal method of canister use, and air sampling filters placed 2 m away from the canisters were used to capture aerosolized CS-derived compounds.

Each air sampling filter consisted of a 37 mm polytetraflouroethylene (PTFE) filter and stainless steel backup pad mounted in an open-faced polystyrene filter cassette (SKC Inc., Eighty Four, PA). Each filter was connected to a portable sampling pump (Gil-Air, Gilian, Wayne, New Jersey) by a 1 m section of Tygon® tubing. Pump flow was set to 4 liters per minute by visual inspection of each pump rotameter. A sample of unheated bulk CS material from an unused canister was also collected for analysis.

*Sample Preparation.* PTFE filters were removed from each cassette using tweezers cleaned in methanol, and were placed in individual 25 mL vials. All samples were placed in a cooler containing dry ice in preparation for a 1 h return trip to the laboratory. Samples were analyzed the following day. An aliquot of 3 mL of dichloromethane was added to each of the filters to extract analytes from the sampling media in preparation for OTGC/MS analysis. The extraction (static mode) of analytes was carried out for 5 min after which time a 10 µl syringe was used to inject 1 µl of each sample into the GC/MS system.

*Gas Chromatography/Mass Spectrometry.* Laboratory analyses to separate and analyze the CS-derived compounds captured on sampling filters were performed using an HP 6890 series gas chromatograph with an HP 5973 quadrupole mass selective detector (Hewlett Packard, Wilmington, DE). The GC was fitted with a J & W Scientific (Folsom, CA) DB-5, 30m x 0.25 mm I.D. column having a film thickness of 0.25 µm. Helium at 1 mL/min was used as the carrier gas. The oven was programmed to increase

from 40 to 160°C at 10°C per minute; 160 to 172°C at 2°C per minute; and 172 to 300°C at 20°C per minute. Samples were analyzed in the splitless injection mode with the injector temperature maintained at 250°C. The mass spectrometer transfer line was kept at 270°C. Electron impact ionization was used (70 eV) and mass spectra were collected over the range of 40-450 m/z. Sample retention characteristics and mass spectra were stored using the HP G1701BA, Version B.00.00 software package.

## RESULTS AND DISCUSSION

Initial and tentative identification of CS-derived compounds was performed by comparing their mass spectra to those of compounds listed in the National Institute of Standards and Technology (NIST) mass spectral library [15]. The probability of obtaining correct library matches for our sample analytes from the NIST mass spectral library was minimal given the uniqueness of the CS-derived compounds and the likelihood that structural isomers were present for a number of the degradation products. Hence, mass spectral interpretation was followed by purchase or synthesis of an authentic standard material in order to confirm peak identity by GC retention time and MS spectrum match.

A chromatogram of bulk material from an unused canister that was not subject to heating showed that a CS isomer (4-chlorobenzylidenemalononitrile) was present in addition to the CS starting material. Besides this minor peak, CS was the only other GC/MS peak observed in unheated canister material. Additional compounds likely derived from the CS starting material were observed after heat-dispersion and capture of analytes. Figure 2-1 shows a chromatogram from the analysis of a solvent-extracted filter

obtained by air sampling during heat-dispersion of CS. CS was the dominant compound present. The mass spectra of CS and the CS-derived compounds detected and identified by retention time and mass spectrum match are given in Table 2-1.

The mass spectral data for peaks (12) and (16) suggested the formation of cyanocinnamonitriles. To determine the location of the cyano group on the benzene ring, standards of 2-cyanocinnamonitrile, 3-cyanocinnamonitrile and 4-cyanocinnamonitrile were prepared (both *cis* and *trans* isomers of each compound). Comparison of the collected samples to the standards indicated that only *cis* and *trans*-2-cyanocinnamonitrile were present in the samples. Formation of 2-cyanocinnamonitrile likely involved homolytic cleavage of the C-Cl bond in CS followed by attack of the resulting radical on the proximal cyano group. Ring opening followed by hydrogen atom abstraction from an environmental source would yield the 2-cyanocinnamonitriles (Figure 2-2). This mechanism precludes formation of other cyanocinnamonitrile isomers. The mechanism for formation of 3-isoquinoline carbonitrile (peak 17) presumably involves formation of the vinyl radical invoked in Figure 2-2. Attack of the radical at the nitrogen of the available cyano group provides the isoquinoline skeleton. Addition of a hydrogen atom yields the corresponding isoquinoline (Figure 2-3). Homolytic C-Cl bond cleavage also likely leads to the formation of benzylidenemalononitrile (peak 10) and 3-quinoline carbonitrile (peak 14) [13].

CS epoxide (peak 8) was poorly resolved from 2-chlorohydrocinnamonitrile (peak 9). Further research is needed to determine if CS epoxide is a sampling artifact. Preliminary data indicate that CS epoxide formation may be facilitated by the presence of unsilanized glass wool found in sorbent tubes or unsilanized glass vials used during

extraction. In addition, work is underway to determine if gas phase salts such as HCN or HCl are released as contaminants during heat dispersion of CS riot control agent.

Identification of the compounds of concern, followed by toxicity assessment, exposure assessment, and risk characterization are the four steps of a baseline human health risk assessment [16]. Further research is warranted to fulfill the remaining health risk assessment process requirements necessary to assess health risks associated with exposure to the compounds we have identified. Previous assessments of human CS exposure have focused on CS alone, not accounting for the CS-derived thermal degradation products [17-23]. A realistic health risk assessment requires the identification of all compounds of concern to which exposure may occur, in addition to the parent compound or starting material.

## ACKNOWLEDGMENTS

We are grateful to United States Army Colonel (retired) Robert Fitz of the Henry Jackson Foundation along with Dr. Howard Vinopal and Mr. Kenneth Williams of the United States Army Center for Health Promotion and Preventive Medicine for providing advice and analytical support necessary in conducting this research.

## REFERENCES


1. Corson, B.B.; Stoughton, R.W., Journal of the American Chemical Society 1928, 50, 2825-2837.
2. Textbook of Military Medicine; Zajtchuk, R., Ed.; Office of the Surgeon General, Department of the Army, United States of America: 1997, p 310.
3. Zebra, E.N.; Ruveda, M.A., Journal of Chromatography 1972, 68, 245-247.
4. Jane, I.; Wheals, B.B., Journal of Chromatography 1972, 70, 151-153.
5. Nowicki, J., Journal of Forensic Science 1981, 27, 3, 704-709.
6. Martz, R.M.; Reutter, D.J.; Lasswell, L.D.I., Journal of Forensic Sciences 1983, 28, 1, 200-207.
7. Tripathi, D.N.; Malhorta, R.C.; Bhattacharya, A., Journal of Chromatography 1984, 315, 417-420.
8. Wils, E.R.J.; Hulst, A.G., Fresenius Zeitschrift fur Analytische Chemie. 1985, 320, 357-360.
9. Wils, E.R.J.; Hulst, A.G., Journal of Chromatography 1985, 330, 379-382.
10. Kaipainen, A.; Kostiainen, O.; Riekkola, M.L., Journal of Microcolumn Separations 1992, 4, 245-251.
11. D'Agostino, P.A.; Provost, L.R., Journal of Chromatography 1995, 695, 1, 65-73.
12. Kokko, M., Journal of Chromatography 1992, 630, 1-2, 231-249.
13. Smith, P.A., Kluchinsky, T.A.; Savage, P.B.; Erickson, R.P.; Lee, A.P.; Williams, K.; Stevens, M.; Thomas, R.J., Journal of the American Industrial Hygiene Association 2000, submitted.
14. Harrison, J.M.; Inch, T.D., Tetrahedron Letters 1985, 22, 679-692.

15. National Institute of Standards and Technology (NIST), NIST/EPA/NIH mass spectral database, standard reference database 1A and the NIST mass spectral search program version 1a. Gaithersburg, MD, 1995.

16. United States Environmental Protection Agency, Risk Assessment Guidance for Superfund, Volume I, Human Health Evaluation Manual (Part A), 1989.

17. Punte, C.L.; Owens, E.J.; Gutentag, P.J., Archives of Environmental Health 1963, 6, 72-80.

18. Beswick, F.W.; Holland, P.; Kemp, K.H., British Journal of Industrial Medicine 1972, 29, 298-306.

19. Cotes, J.E., Quarterly Journal of Experimental Physiology 1972, 57, 199-206.

20. Elskamp, D.M.W., Toxic properties of CN and CS 1976, Department of the Army: Netherlands.

21. Holland, P.; White, R.G., British Journal of Dermatology 1972, 86, 150-154.

22. Jones, G.R.N., Nature 1972, 235, 257-261.

23. Weigand, D., Military Medicine 1969, 134, 437-440.

Table 2-1. The Mass Spectra of CS and CS-derived Compounds

| No. | Compound | Ret. Time (min) | m/z (% relative intensity) |
|---|---|---|---|
| 1 | 2-chlorobenzaldehyde | 8.504 | 50 (20), 51 (14), 74 (13), 75 (24), 76 (23), 77 (19), 111 (37), 112 (12), 113 (16), 139 (100), 140 (70), 141 (44), 142 (23) |
| 2 | 2-chlorobenzonitrile | 9.279 | 75 (19), 76 (13), 84 (12), 102 (33), 137 (100), 139 (32) |
| 3 | quinoline | 10.297 | 51 (13), 76 (12), 102 (24), 103 (10), 128 (21), 129 (100), 130 (13) |
| 4 | 2-chlorobenzylcyanide | 10.930 | 89 (18), 115 (20), 116 (100), 117 (11), 151 (34), 153 (9) |
| 5 | 1,2-dicyanobenzene | 11.410 | 75 (12), 101 (18), 128 (100), 129 (9) |
| 6 | 3-(2-chlorophenyl)propynenitrile | 11.981 | 99 (18), 100 (10), 126 (18), 161 (100), 162 (11), 163 (34) |
| 7 | *cis* 2-chlorocinnamonitrile | 12.233 | 50 (10), 51 (10), 75 (17), 100 (11), 101 (20), 127 (10), 128 (100), 136 (10), 163 (53), 165 (18) |
| 8 | 2, 2-dicyano 3-(2-chlorophenyl) oxirane | 12.385 | 44 (15), 50 (25), 51 (19), 74 (19), 75 (35), 76 (20), 77 (22), 89 (66), 111 (27), 119 (19), 139 (40), 140 (40), 141 (100), 149 (34), 153 (26), 204 (22) |
| 9 | 2-chlorohydrocinnamonitrile | 12.437 | 89 (16), 125 (100), 127 (35), 165 (21) |
| 10 | benzylidenemalononitrile | 13.241 | 50 (14), 51 (14), 75 (10), 76 (17), 100 (14), 103 (64), 127 (82), 128 (12), 153 (10), 154 (100), 155 (13) |
| 11 | *trans* 2-chlorocinnamonitrile | 13.412 | 50 (11), 74 (10), 75 (15), 77 (11), 100 (9), 101 (20), 127 (9), 128 (100), 129 (11), 163 (58), 165 (19) |
| 12 | *cis* 2-cyanocinnamonitrile | 13.635 | 50 (10), 75 (13), 76 (14), 100 (13), 127 (100), 128 (14), 153 (11), 154 (95), 155 (12) |
| 13 | 2-chlorobenzylmalononitrile | 14.130 | 89 (16), 125 (100), 127 (32), 190 (8) |
| 14 | 3-quinoline carbonitrile | 14.192 | 100 (10), 127 (32), 154 (100), 155 (12) |
| 15 | 2-chlorobenzylidenemalononitrile | 15.081 | 75 (15), 76 (12), 99 (13), 100 (12), 126 (22), 137 (12), 153 (100), 154 (14), 161 (18), 188 (47), 190 (16) |
| 16 | *trans* 2-cyanocinnamonitrile | 15.775 | 50 (10), 75 (12), 76 (15), 100 (14), 127 (100), 128 (13), 153 (12), 154 (96), 155 (11) |
| 17 | 3-isoquinoline carbonitrile | 16.622 | 127 (35), 154 (100), 155 (12) |
| 18 | 4-chlorobenzylidenemalononitrile | 16.750 | 50 (13), 51 (10), 74 (10), 75 (19), 76 (16), 99 (13), 100 (13), 102 (10), 126 (21), 137 (25), 153 (100), 154 (12), 161 (30), 163 (10), 188 (78), 189 (10), 190 (25) |

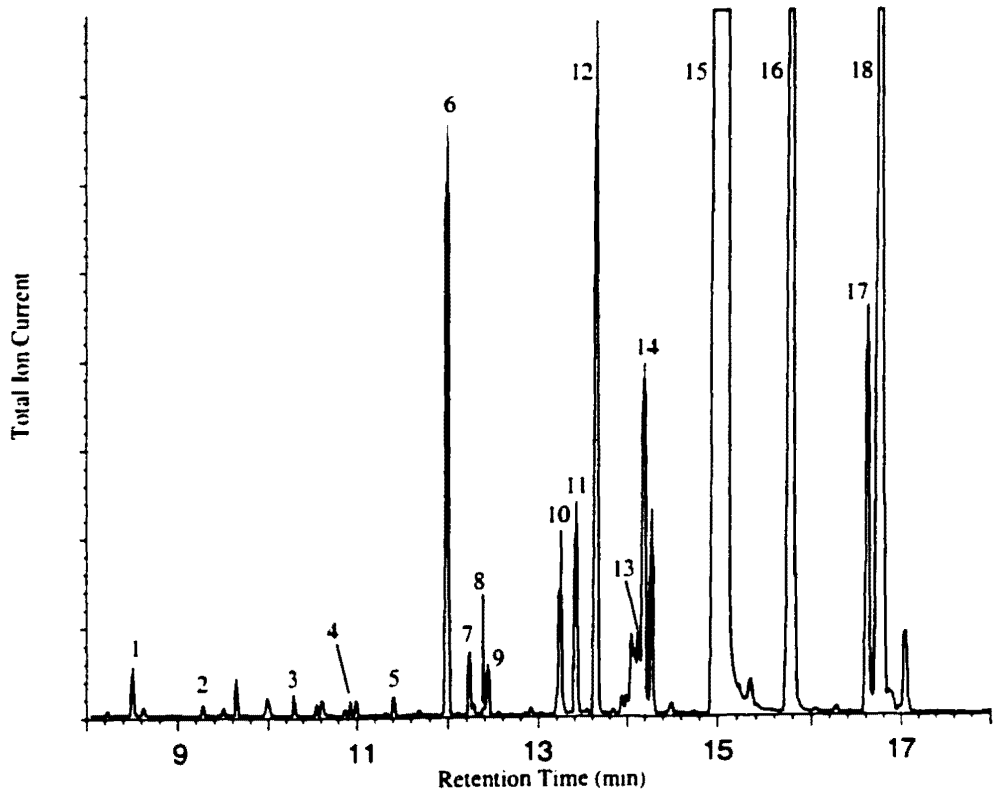



**Figure 2-1.** Chromatogram of dichloromethane-extracted filter obtained by air sampling during heat-dispersal of CS from law enforcement type canisters. Table 2-1 gives peak identities.

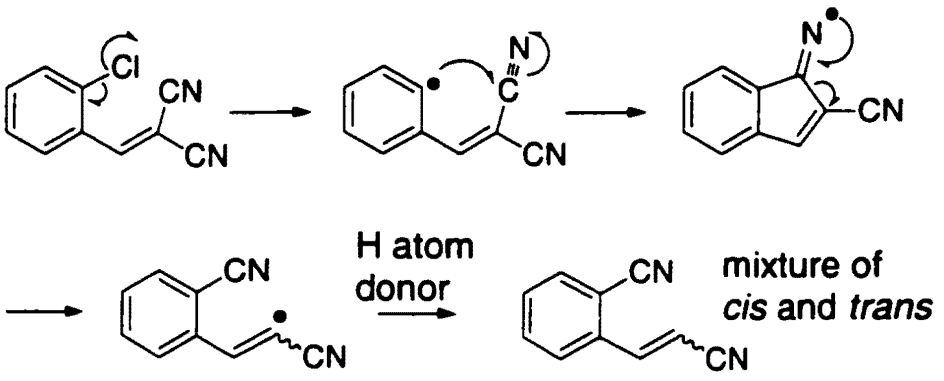



**Figure 2-2.** Proposed mechanism for the formation of *cis* and *trans* 2-cyanocinnamonitrile from CS.

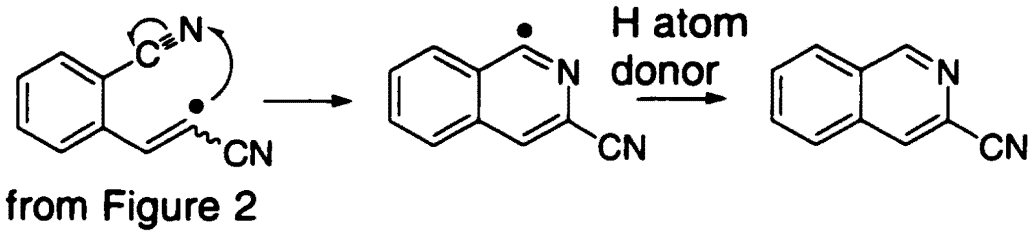



**Figure 2-3.** Proposed mechanism for the formation of 3-isoquinoline carbonitrile from CS.

# CHAPTER 3

## Liberation of Hydrogen Cyanide and Hydrogen Chloride during High Temperature Dispersion of CS Riot Control Agent

[1]Timothy A. Kluchinsky, Jr., [2]Paul B. Savage, [1]Robert Fitz, *[1]Philip A. Smith [1]Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, Bethesda MD, [2]Brigham Young University, Department of Chemistry and Biochemistry, Provo UT, *Corresponding author.

**ABSTRACT:** High temperature dispersion of the riot control agent 2-chlorobenzylidenemalononitrile (CS) has previously been shown to produce a number of organic thermal degradation products through rearrangements and loss of cyano and chlorine substituents present on the parent CS compound. Until now, the possibility that HCN and HCl may also be produced during high temperature CS dispersion has not been examined. We collected air samples to detect HCN and HCl as air contaminants released during high temperature CS dispersion indoors. Sampling and analysis using the National Institute of Occupational Safety and Health methods 7904 and 6010 (modified) for HCN, and 7903 for HCl, showed evidence that both compounds were present in air samples collected. A re-assessment of human health risks associated with exposure to CS riot control agent dispersed at high temperature should be conducted, and should consider the full range of contaminants produced during the dispersion process.

**KEY WORDS:** 2-chlorobenzylidenemalononitrile; degradation product; hydrogen cyanide; hydrogen chloride.

This manuscript has been completed in partial fulfillment of the degree of Doctor of Public Health, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland. 

## INTRODUCTION

High temperature dispersion of 2-chlorobenzylidenemalononitrile (CS riot control agent) has been shown to form at least sixteen organic thermal degradation products [1, 2]. The presence of 3-(2-chlorophenyl)propynenitrile as one of the CS-derived compounds suggested loss of hydrogen cyanide (HCN) from the CS parent compound (Figure 3-1).

Additional degradation products observed in the previous work cited [1, 2] (benzylidenemalononitrile, *cis* and *trans* isomers of 2-cyanocinnamonitrile, 3-quinolinecarbinitrile, and 3-isoquinolinecarbonitrile) appear to result from loss of chlorine radical. Eventual hydrogen atom abstraction by the chlorine radical from an environmental source would produce hydrogen chloride (HCl) and an additional radical species.

HCN is an extremely toxic, colorless, gas with a characteristic almond odor. Exposure to HCN can result in a potentially fatal histotoxic hypoxia condition caused by inhibition of cytochrome oxidase. This condition reduces the ability of tissues to properly utilize oxygen necessary for tissue respiration [3]. HCl is a colorless,

nonflammable gas that affects the eyes and mucous membranes. Exposure to high concentrations of HCl can result in laryngitis, bronchitis, and pulmonary edema [4].

## MATERIALS AND METHODS

*Heat dispersion of CS*

Two continuous discharge, Type 3, CS canisters purchased from Armor Holdings Inc. (Jacksonville, FL) were ignited through the normal mechanism (release of mechanically-actuated fuse) dispersing CS and degradation products from the center floor area of an enclosed 240 $m^3$ chamber used by law enforcement agencies for riot control agent training. The chamber was well ventilated prior to CS dispersion and sampling.

Four samples collected during CS dispersion as described above were submitted with four field blanks for analysis using the National Institute of Occupational Safety and Health (NIOSH) method 7904 for HCN. Sampling was conducted using the recommended two stage sampling train: 0.8 μm polyvinyl chloride filter mounted inside an open-faced filter cassette (SKC Inc., Eighty Four, PA) to capture cyanide salts followed by an impinger containing 15 mL of 0.1 normal potassium hydroxide solution to trap hydrogen cyanide gas [5]. Personal sampling pumps were used (Escort® ELF, Charleston, SC) and calibrated before and after sampling. The "pre" and "post" sampling flow rates of each pump differed by less than 5 % and were between 1.00 and 1.09 L/min. Upon completion of sample collection, filter cassettes were capped and the contents of each impinger were transferred to individual 40 mL vials. Samples were shipped to Galson Laboratories (East Syracuse, NY) and analyzed within five days.

For analysis of these samples, filters were transferred to 50 mL centrifuge tubes where 25 mL of 0.1 N potassium hydroxide (KOH) was added and allowed to stand with occasional shaking for 30 minutes. The basic impinger liquid samples were transferred to 25 mL volumetric flasks and brought to volume using 0.1 N KOH. Both filter and impinger samples were analyzed at room temperature using a Thermal Orion cyanide ion specific electrode (Beverly, MA) and a Thermal Orion single junction reference electrode connected to a Thermal Orion 420A meter. Sulfide ion, which has the potential to irreversibly poison the $CN^-$ electrode, was shown to be absent using lead acetate paper. The data were electronically stored by manual entry into a Microsoft Excel spreadsheet.

Four samples and four blanks were collected to attempt detection of HCl, at the same time as the preceding samples, and from the same location. NIOSH method 7903 sampling procedures were followed [6]. Solid sorbent tubes (washed silica gel, SKC Inc., Eighty Four, PA) were used to capture hydrogen chloride gas with personal sampling pumps (Gilian, St. Louis, MO), which were calibrated before and after sampling. The "pre" and "post" sampling flow rates of each pump differed by less than 7 % for three of the four pumps; however, the flow rates of the fourth pump differed greatly, indicative of a faulty pump, and its corresponding sample was not included in the results. Flow rates were between 0.20 and 0.22 L/min (excluding the faulty pump). The sorbent tubes were capped and transferred to the Directorate of Laboratory Sciences, United States Army Center for Health Promotion and Preventive Medicine (Edgewood, MD) where they were analyzed within twenty-one days using ion chromatography (IC) as recommended by NIOSH method 7903.

For analysis of the preceding HCl samples, the front and back washed silica gel sections of each tube were removed and desorbed separately with 10 mL of deionized water. Samples were swirled and allowed to stand for one hour. Laboratory analyses were performed using a Dionex DX 500 ion chromatograph (Sunnyvale, CA) equipped with a Dionex AS40 autosampler with 5.0 mL injection vials with filter caps and cassettes, Dionex LC20 chromatography enclosure with air actuated rotary injection valve and 50 μL injection loop, Dionex GP-40 gradient pump, Dionex AS4A anion separator column, Dionex AG4A anion separator guard column, Dionex CD-20 conductivity detector and Dionex DS3 conductivity cell. The flow rate of the eluent mobile phase, consisting of 1.7 mM sodium bicarbonate and 1.8 mM sodium carbonate, was 2 mL/min. Data were managed using Dionex Peaknet software version 5.1.

Two additional HCN samples were collected on a subsequent date during CS dispersion as already described, and were submitted with two field blanks for analysis using a modified version of NIOSH method 6010. Sampling was conducted using the method's recommended two stage sampling train: a sorbent tube containing a 0.8 μm mixed cellulose ester filter to capture cyanide salts, followed by soda lime sorbent to trap hydrogen cyanide gas (SKC Inc., Eighty Four, PA) [7]. Personal sampling pumps (Escort® ELF, Charleston, SC) were calibrated before and after sampling. The "pre" and "post" sampling flow rates of each pump differed by less than 4% and were between 0.49 and 0.51 L/min. Samples were shipped to Wisconsin Occupational Health Laboratory (Madison, WI) and analyzed within three days by IC with DC amperometric detection, rather than by spectrophotometry as specified by the method.

These samples were prepared by removing the filter and glass wool sections of each tube and desorbing them in 10 mL of 0.1 N sodium hydroxide. The front and back soda lime sections of each tube were removed and desorbed separately in 10 mL of deionized water. Samples were shaken and allowed to stand for one hour. Laboratory analyses were performed using a Dionex DX 500 ion chromatograph (Sunnyvale, CA) equipped with a Dionex AS40 autosampler with 0.5 mL injection vials with filter caps and cassettes, Dionex LC20 chromatography enclosure with air actuated rotary injection valve and 50 µL injection loop, Dionex GP-50 gradient pump, Dionex AS7 anion separator column, Dionex AG7 anion separator guard column, and Dionex ED-40 electrochemical detector and cell using silver working electrode, silver chloride reference, and 0.00 V applied. The flow rate of the eluent mobile phase, consisting of 0.5 M sodium acetate, 0.1 M sodium hydroxide, and 0.5 % ethylene diamine, was 1 mL/min. Data were managed using Dionex Peaknet software version 5.1. The laboratory also spiked a sample with $CN^-$ for retention time comparison.

## RESULTS

### *HCN*

Sampling and analysis using NIOSH method 7904 showed HCN to be present in all four impinger samples (Table 3-1) at levels near or above the 4.7 ppm threshold limit value ceiling (TLV-C) adopted by the American Conference of Governmental Industrial Hygienists (ACGIH) [8] and the 4.7 ppm recommended exposure limit short-term exposure limit (REL-STEL) published by NIOSH [9]. The 10 ppm permissible exposure limit time weighted average (PEL-TWA) mandated by the Occupational Safety and

Health Administration (OSHA) was not exceeded [10]. Particulate cyanide was below or slightly above the method's detectable levels. No HCN was detected in field blanks. Sampling and analysis using NIOSH method 6010 (modified for analysis by IC) showed HCN to be present in both sorbent tube samples collected within the exposure chamber (Table 3-1). The concentrations of HCN detected using this method exceeded the TLV-C and REL-STEL. Again, the particulate cyanide values were below or slightly above the method's detectable levels. No HCN was detected in field blanks. Figure 3-2 provides a comparison of IC chromatograms from a field blank, one of our samples, and a sample spiked with $CN^-$ for retention time comparison.

*HCl*

Sampling and analysis using NIOSH method 7903 detected chloride in all three samples collected. The values obtained were well below available standards for HCl [8] (Table 3-1). No chloride was detected in field blanks submitted for analysis.

## DISCUSSION

The high temperature process typical of the dispersion of CS riot control agent from hand-tossed canisters has been shown to be capable of producing numerous degradation products [1, 2]. Previous to the work by Smith *et al.* [1] and Kluchinsky *et al.* [2], little evidence acknowledging the potential production of organic thermal degradation products resulting from that dispersion process existed in the peer-reviewed literature. Prior to our work here, there is no evidence in the literature that high temperature CS dispersion had been examined for its potential to produce the gas phase

analytes of the present study. Incomplete data concerning chemical species to which people are exposed under real-life conditions will lead to incomplete risk assessments for exposures.

After examining the range of contaminants made airborne following high temperature CS dispersion [1, 2] and the gas phase salt type compounds studied here, it is obvious that the risks posed to the general public from use of CS dispersed at high temperatures should be re-evaluated. This is especially important if there is a need to use CS riot control agent dispersed at high temperature in enclosed areas.

The levels of HCN observed in our air samples were near or above ACGIH ceiling and NIOSH short-term exposure limit standards [8,9]. The OSHA 8-hour time weighted average for HCN is a poor choice for comparison with our samples as the exposure time was about 3 minutes rather than 480 minutes. The toxicity of HCN is characterized by a relatively steep dose response curve with rapid onset of a potentially severe response as hazardous concentrations are encountered. The occupational exposure limits cited [8-10] are generally considered to be useful in protecting the health of workers who are considered relatively healthy adults. The possibility that members of the general public may be exposed to HCN during law enforcement operations provides impetus for further study of this issue, especially relating to concentrations attained under a variety of circumstances. Alternative CS riot control agent dispersion methods that do not rely on heating (or that disperse at lower temperatures) may merit consideration.

Based upon the Table 3-2 HCN concentrations observed using a modified NIOSH method 1610, the NIOSH immediately dangerous to life and health (IDLH) HCN concentration of 50 ppm [11] could conceivably be exceeded, in addition to the TLV-C

and REL-STEL, by dispersing multiple CS riot control agent canisters inside a small room.

## CONCLUSION

Previous work to identify organic degradation products resulting from high temperature dispersion of the riot control agent CS suggested that airborne HCN and HCl may also result from such dispersion. We collected air samples during high temperature dispersion of CS from riot control type canisters indoors and analyses showed HCN and chloride (likely as HCl), to be present in air samples collected. Analysis of additional samples using the NIOSH method 6010-modified to include ion chromatography confirmed the presence of HCN as an air contaminant produced during high temperature dispersion. The decision making process in scenarios where CS use may be warranted should account for the presence of contaminants other than CS, including those studied here. The human health risks associated with its use (especially in enclosed spaces) should be re-assessed so that exposure to HCN and HCl, as well as other high temperature dispersed CS-derived thermal degradation compounds, are included in the assessment.

## ACKNOWLEDGMENTS

We are grateful to the Wisconsin Occupational Health Laboratory who provided our research group with an alternative method for HCN and particulate cyanide analysis. We are also very appreciative of the United States Army Center for Health Promotion and Preventive Medicine-North for their support and the sampling efforts provided by

United States Army Environmental Science Officers Captain Tara Hall, Captain Mark Hayden, and Captain Gregory Kimm in conducting this research.

## REFERENCES


1. Smith, P.A., T.A. Kluchinsky, Jr., P.B. Savage, R.P. Erikson, A.P. Lee, K. Williams, M. Stevens, and R.J. Thomas: Traditional sampling with laboratory analysis and solid phase microextraction sampling with field gas chromatography/mass spectrometry by military industrial hygienists. *Journal of the American Industrial Hygiene Association* submitted (2000).
2. Kluchinsky, T.A.Jr., P.B. Savage, M.V. Sheely, R.J. Thomas, and P.A. Smith: Identification of CS-derived compounds formed during heat-dispersion of CS riot control agent. *Journal of Microcolumn Separations* accepted (2001).
3. Agency for Toxic Substances and Diseased Registry (ATSDR): *Toxicological Profile for Cyanide*, Atlanta, GA: ATSDR, 1997.
4. Ron, W.N.: *Environmental and Occupational Medicine*. Boston, MA: Little & Brown, 1983. p. 275.
5. National Institute of Occupational Safety and Health (NIOSH): Method 7904, Cyanides, aerosol and gas. In *NIOSH Manual of Analytical Methods*. Cincinnati, OH: NIOSH, 1994.
6. National Institute of Occupational Safety and Health (NIOSH): Method 7903, Acids, inorganic. In *NIOSH Manual of Analytical Methods*. Cincinnati, OH: NIOSH, 1994.
7. Wisconsin Occupational Health Laboratory (WOHL): Method NIOSH 6010-modified (IC), Hydrogen cyanide. Madison, WI: WOHL, 1997.
8. American Conference of Governmental Industrial Hygienists (ACGIH®), *Guide to Occupational Exposure Values 2000*. Cincinnati, OH: ACGIH, 2000.

9. National Institute of Occupational Safety and Health (NIOSH), *NIOSH Pocket Guide to Chemical Hazards* (NIOSH pub. no. 97-140). Cincinnati, OH: NIOSH, 1997.

10. "Air Contaminants: Final Rule," *Code of Federal Regulations*, Title 29, Part 1910. 1993.

11. Ludwig, H.R., S.G. Cairelli, and J.J. Whalen: Eds. *Documentation for Immediately Dangerous to Life or Health Concentrations* (NTIS pub. no. PB-94-195-047). Springfield, VA: NTIS, 1994.

**Table 3-1. Sampling results for hydrogen cyanide (HCN), particulate cyanide ($CN^-$), and hydrogen chloride (HCl)**

| Method | Compound | Sample Number | Concentration[1] |
|---|---|---|---|
| NIOSH 7904 | HCN | 1 | 5.1 |
| | | 2 | 5.4 |
| | | 3 | 3.9 |
| | | 4 | 4.2 |
| | $CN^-$ | 1 | 0.25 |
| | | 2 | 0.29 |
| | | 3 | <0.2[2] |
| | | 4 | <0.2[2] |
| NIOSH 6010[3] | HCN | 1 | 15 |
| | | 2 | 10 |
| | $CN^-$ | 1 | <=6[2] |
| | | 2 | <0.6[4] |
| NIOSH 7903 | HCl | 1 | 0.8 |
| | | 2 | 0.7 |
| | | 3 | 1.1 |

[1]Expressed in parts per million (ppm) for HCN and HCl and milligrams per cubic meter ($mg/m^3$) for $CN^-$.

[2]Less than the method's specified level of quantitation.

[3]Method modified to include ion chromatography with amperometric detection rather than spectrophotometry.

[4]Less than the method's specified level of detection.

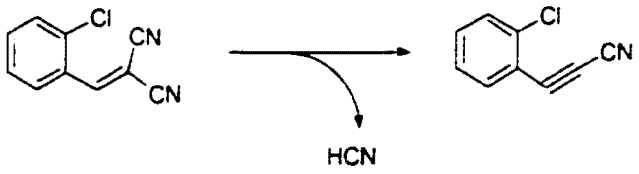



**Figure 3-1.** HCN loss from CS giving the thermal degradation product 3-(2-chlorophenyl)propynenitrile observed in previous work.

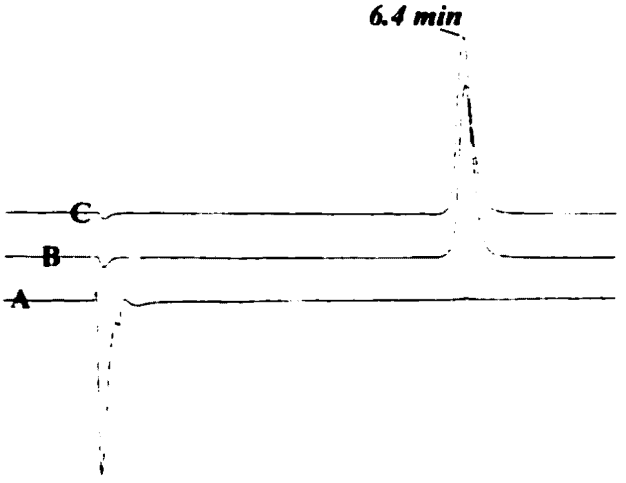



**Figure 3-2.** IC chromatograms from analysis of **(A)** field blank; **(B)** sample collected in the field; and **(C)** $CN^-$ spiked sample. The single peak in the field sample matches the $CN^-$ spike sample retention time.

# CHAPTER 4

## Formation of 2-chlorobenzylidenemalononitrile (CS Riot Control Agent) Thermal Degradation Products at Elevated Temperatures

[1]Timothy A. Kluchinsky, Jr., [2]Michael V. Sheely, [3]Paul B. Savage, *[1]Philip A. Smith
[1]Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, Bethesda MD, [2]United States Army Center for Health Promotion and Preventive Medicine, Aberdeen MD, [3]Brigham Young University, Department of Chemistry and Biochemistry, Provo UT, *Corresponding author.

## ABSTRACT

2-chlorobenzylidenemalononitrile (CS riot control agent) has previously been shown to produce a number of thermal degradation products when dispersed at high temperature. We hypothesized that these CS-derived compounds are formed by input of energy from heating during the dispersion process. In this work, we identified organic CS-derived compounds formed from purified CS subjected to temperatures ranging from 300-900°C in an inert atmosphere with analysis of gas phase tube furnace effluent by gas chromatography and mass spectrometry. We conclude that the production of many CS-derived compounds previously observed during high-temperature dispersion is likely to be heat related. If CS-derived compounds produced during heat-dispersion of CS canisters prove to be a public health hazard, alternative dispersion methods may be warranted.

**KEY WORDS:** CS riot control agent, thermal degradation products, tube furnace.

This manuscript has been completed in partial fulfillment of the degree of Doctor of Public Health, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the United States Department of Defense or the Uniformed Services University of the Health Sciences.



## INTRODUCTION

Law enforcement personnel, correctional officers, rioters, and bystanders may be exposed to 2-chlorobenzylidenemalononitrile (CS) riot control agent. Also, military combat training may include exposure to CS dispersed by heat. Several studies evaluating CS toxicity have concluded that CS elicits no harmful effects when used properly other than its intentional short-duration incapacitating effect [1-4]. Kluchinsky *et al.* and Smith *et al.* identified the presence of eighteen organic [5-6] and two gas phase salt type [7] compounds as a result of dispersion of CS at high temperature from small hand-tossed "riot control" type canisters. Acute toxicity studies establishing lethal dose/concentration fifty percent values (LD50/LC50) currently exist for only 8 of these twenty compounds [8-26]. In addition to the need to identify these compounds and to understand their toxicity, knowledge of the temperature ranges associated with their formation is also important. If CS-derived compounds produced during heat-dispersion of CS canisters prove to be a public health hazard, alternative dispersion methods may be warranted.

## EXPERIMENTAL

*Materials.* CS obtained from Aldrich (Milwaukee, WI) was recrystallized in cold ethyl acetate (Fisher Scientific, HPLC grade, Fairlawn, NJ) and confirmed to be chromatographically pure with a trace of the CS isomer 4-chlorobenzylidene-malononitrile also present. 200 mg of CS was dissolved in 1 mL of dichloromethane (Burdick & Jackson, capillary GC/GC-MS grade, Muskegon, MI) to create a stock solution to be used in heating experiments. Continuous discharge, Type 3, CS canisters were purchased from Armor Holdings Inc. (Jacksonville, FL).

*Analytical Standards.* Analytical standards were purchased from Aldrich (Milwaukee, WI) for CS (97%), 2-chlorobenzaldehyde (99%), 2-chlorobenzonitrile (98%), quinoline (98%), 2-chlorobenzylcyanide (98%), 1,2-dicyanobenzene (98%), 2-chlorohydrocinnamonitrile (99%), benzylidenemalonontrile (98%), 3-quinoline carbonitrile (99%), 3-isoquinoline carbonitrile (99%) and 4-chlorobenzylidene-malononitrile (unknown purity). Standards for 2-cyanocinnamonitrile (*cis* and *trans*), 2-chlorocinnamonitrile (*cis* and *trans*), 2-chlorobenzylmalononitrile (dihydro CS), 3-(2-chlorophenyl)propynenitrile, and 2,2-dicyano-3-(2-chlorophenyl)oxirane (CS epoxide) [5,27], were synthesized as they were not available from commercial sources. For all standards synthesized, structures were confirmed using $^{1}H$ NMR, $^{13}C$ NMR, and high resolution-fast atom bombardment MS in addition to the GC/MS methods reported here.

*Field Dispersion Temperature of CS Canisters.* The canisters used in our study contain a mixture of pelletized CS, potassium chlorate, magnesium carbonate, nitrocellulose, and sugar (specific sugar not specified by manufacturer) [28]. A mechanically actuated fuse ignites the mixture resulting in dispersion of CS. The

temperatures of three CS canisters were measured during dispersion of their contents using a Hotmux Data Logger (DCC Corporation, Pennsauken, NJ) with collection of digitized temperature data through a serial port connection to a laptop personal computer using proprietary Hotmux software. These data were used to determine the temperatures used during our tube furnace heat experiments.

A hole was drilled in the side of each CS canister into the pelletized CS. A K type wire with a 24 gauge thermocouple bead on the end was placed inside the resulting hole to a depth of approximately 2 cm, and was covered with aluminum tape. The other end of the wire was connected to the data logger which used an RS-232 cable to interface with the computer.

*Tube Furnace Experiments.* The system used is shown in Figure 4-1. 20 μL of the CS/dichloromethane stock solution was spiked onto a porcelain combustion boat (Coors 08, Golden, CO). The boat was placed inside the quartz tube by removing the rubber septum from the end of the tube. The solvent was allowed to evaporate at room temperature from the boat, the rubber septum of the quartz tube was refastened, and the system was purged with nitrogen to remove air prior to heating. A pushrod extending through a small hole punctured in the rubber septum was used to advance the boat to the center of the 107 cm x 2.5 cm I.D. quartz tube partially enclosed in a Fisher Scientific Isotemp® model 189 Tube Furnace (Fair Lawn, NJ). The system was operated at temperatures of 300, 500, 700, and 900°C as displayed on the instrument's temperature gauge. The tube furnace's ability to register the correct temperature was confirmed at each temperature reading using the data logger ($r^2$=0.99, slope=0.99). CS and thermal degradation products formed at these temperatures were swept through the quartz tube by

nitrogen at a flow rate of 480-595 mL/min and trapped in an impinger containing 20 mL of dichloromethane. The "pre" and "post" flow rates of each run differed by less than 9 %. Three replicate samples were produced at each temperature. A residence time range of 10-12 seconds was derived based on the carrier gas flow rate range and the volume of heated space traversed (97 mL) through the quartz tube by the rapidly volatilized CS and resulting degradation products. The length of quartz tube traversed was calculated from the center of the tube furnace where the boat was placed, to the exit end of the tube furnace (19 cm).

The same analytical procedures were followed for a blank sample except it was spiked with neat dichloromethane rather than the dichloromethane stock solution. An unheated sample of CS/dichloromethane stock solution was also analyzed.

*Sample Analysis by Gas Chromatography/Mass Spectrometry.* The contents of each impinger were immediately removed and brought to a volume of 20 mL by adding dichloromethane. An aliquot of each sample was placed into separate 1.5 mL vials in preparation for GC/MS analysis.

Laboratory analyses to separate and analyze the CS-derived compounds captured in impingers containing dichloromethane were performed within one hour using an HP 5890 series gas chromatograph (GC) equipped with an HP 7673 auto sampler using an injection volume of 1 μL, and an HP 5971A quadrupole mass selective detector (Hewlett Packard, Wilmington, DE). The GC was fitted with a Zebron (Torrance, CA) ZB-5, 30m x 0.25 mm I.D. column having a film thickness of 0.25 μm. Helium at 0.9 mL/min was used as the carrier gas. The GC oven was held at 40°C for 1 min, then programmed to increase from 40 to 200°C at 10°C per minute, and held at 200°C for 2 min. Samples

were analyzed in the splitless injection mode with the injector temperature maintained at 250°C. The mass spectrometer transfer line was kept at 255°C. Electron impact ionization was used (70 eV) and mass spectra were collected over the range of 35-300 m/z. Sample retention characteristics and mass spectra were stored using the HP G1701BA, Version B.00.00 software package. Identification of chromatogram peaks with signal-to-noise ratio greater than 3:1 was by GC retention time and MS mass spectrum match. Identification of CS-derived compounds not exhibiting signal-to-noise ratio greater than 3:1 was by GC retention time and examination for extracted ion chromatogram mass spectra of ions known to be important in the mass spectrum of each respective analyte.

## RESULTS

*Field Dispersion Temperature of CS Canisters.* The data logger system used to measure the internal temperature of three CS canisters during dispersion provided a mean maximum temperature of 798°C (95% CI = 749°C, 846°C).

*Tube Furnace Experiments.* No degradation products were present in the blank sample spiked with neat dichloromethane followed by the sample heating, collection, and analysis procedures discussed in the experimental section. 2-chlorobenzaldehyde was the only degradation product observed during analysis of an unheated sample spiked with CS/dichloromethane stock solution. Results of the laboratory tube furnace experiments are listed in Table 4-1. 2-chlorobenzaldehyde (1), 2-chlorobenzylmalononitrile (13), CS (15), and 4-chlorobenzylidenemalononitrile (18) were observed at 300°C and 500°C; 2-chlorobenzaldehyde (1), 2-chlorobenzonitrile (2), 1,2-dicyanobenzene (5), 3-(2-chloro-

phenyl)propynenitrile (6), *cis* 2-chlorocinnamonitrile (7), benzylidenemalononitrile (10), *trans* 2-chlorocinnamonitrile (11), *cis* 2-cyanocinnamonitrile (12), 2-chlorobenzylmalononitrile (13), 3-quinoline carbonitrile (14), CS (15), 3-isoquinoline carbonitrile (17), and 4-chlorobenzylidenemalononitrile (18) were observed at 700°C; and 2-chlorobenzonitrile (2), quinoline (3), 1,2-dicyanobenzene (5), 3-(2-chlorophenyl)-propynenitrile (6), 2,2-dicyano-3-(2-chlorophenyl)oxirane (8), benzylidenemalononitrile (10), *trans* 2-chlorocinnamonitrile (11), *cis* 2-cyanocinnamonitrile (12), 3-quinoline carbonitrile (14), CS (15), and 3-isoquinoline carbonitrile (17) were observed at 900°C. The formation of 2,2-dicyano-3-(2-chlorophenyl)oxirane (8) observed in two of three samples at 900°C is likely an artifact produced by a small amount of oxygen present in the system.

Several of the compounds previously observed in field samples collected from CS riot control canisters [5] were not observed during these laboratory heating experiments. Also, extracted ion chromatogram mass spectra did not show their important MS ions at the retention times observed with the authentic standards. These compounds were 2-chlorobenzylcyanide (4), 2-chlorohydrocinnamonitrile (9), and *trans* 2-cyanocinnamonitrile (16).

*Health Concerns.* A potential health threat exists regarding formation of the compounds *cis* 2-cyanocinnamonitrile (12), 3-quinoline carbonitrile (14), and 3-isoquinoline carbonitrile (17) which are likely produced through free radical mechanisms [5-6]; and 3-(2-chlorophenyl)propynenitrile (6), which is likely formed as a result of a loss of HCN from CS [7]. Our data revealed that *cis* 2-cyanocinnamonitrile (12) was present at 700°C; and 3-quinoline carbonitrile (14) and 3-isoquinoline carbonitrile (17) at

700-900°C. The compound 3-(2-chlorophenyl)propynenitrile (6) also appeared at 700-900°C. It is likely that hydrogen cyanide (HCN) levels increased as well because it appears to be formed as a result of the loss of cyanide from the CS molecule in the formation of 3-(2-chlorophenyl)propynenitrile (6) [7]. The mass spectra of CS-derived compounds observed from temperatures of 300-500°C do not show peaks for 127, 154, or 161 *m/z* ion current, except for CS and its isomer 4-chlorobenzylidenemalononitrile. This provides evidence that compounds thought to possess free radical intermediates (*cis* 2-cyanocinnamonitrile, 3-quinoline carbonitrile, and 3-isoquinoline carbonitrile) are not formed under the conditions present in the system at these low temperatures, and suggests little or no formation of 3-(2-chlorophenyl)propynenitrile resulting in the release of HCN. These results lead us to assume that thermal dispersion of CS canisters at a reduced temperature may be less likely to produce these CS degradation products.

One must use caution when attempting to generalize the products formed in our research to those produced during the actual heat-dispersion of the contents of a CS canister (CS, potassium chlorate, magnesium carbonate, nitrocellulose, sugar) [28] to air. For instance, residence time at a given temperature will affect the formation of thermal degradation products. The residence time for CS molecules at high temperature inside a canister during dispersion is dynamic, complicated, and undefined. For consideration of residence time in our laboratory experiments, we assumed a nearly instantaneous vaporization of spiked CS when placed into the tube furnace (the CS is fairly evenly distributed on a combustion boat having a low thermal mass), and a steady movement of CS and CS-derived compounds in carrier gas through the quartz tube and subsequently into the impinger, with a defined flow rate at all temperatures studied.

## CONCLUSION

We varied tube furnace temperature and standardized the residence time for CS at high temperature, inert gas flow rate, and quantity of CS riot control agent, while thermally degrading CS to investigate the effect of temperature on the production of organic CS-derived compounds. We conclude that degradation products formed as a result of heating CS in a temperature-dependent fashion. These findings should be considered when deploying or thermally disposing of CS riot control agent as the health hazards associated with the production of many such CS-derived compounds have not been adequately evaluated. Formation of the compounds *cis* 2-cyanocinnamonitrile, 3-quinoline carbonitrile, and 3-isoquinoline carbonitrile is of interest as they are likely produced through free radical mechanisms [5-6]. Also, formation of 3-(2-chlorophenyl)propynenitrile from CS likely results in the loss of HCN and measurable airborne concentrations of this compound.

## ACKNOWLEDGMENTS

We are grateful to Colonel James S. Little, Dr. Howard Vinopal, and Mr. Kenneth Williams of the United States Army Center for Health Promotion and Preventive Medicine for providing analytical support necessary to complete this research.

## REFERENCES


1 B. Ballantyne, *Medical Annuls*, (1977/1978) 7-41.

2 F.W. Beswick, *Human Toxicology*, 2 (1983) 247-256.

3 B.L. Danto, *American Journal of Forensic Medical Pathology*, 8 (1987) 317-322.

4 H. Himsworth, London, England, Her Majesty's Stationary Office (1971).

5 T.A. Kluchinsky, Jr, P.B. Savage, M.V. Sheely, R.J. Thomas, P.A. Smith, *Journal of Microcolumn Separations*, accepted (2001)

6. P.A. Smith, T.A. Kluchinsky, Jr., P.B. Savage, R.P. Erickson, A.P. Lee, K. Williams, M. Stevens, R.J. Thomas, *Journal of the American Industrial Hygiene Association*, submitted (2000).

7. T.A. Kluchinsky, Jr., P.B. Savage, R. Fitz, P.A. Smith, *Journal of the American Industrial Hygiene Association*, submitted (2001).

8. Environmental Protection Agency, U.S., Unpublished Test Data Report, *Letter from Eastman Kodak Company to USEPA Submitting Enclosed Toxicity and Health Summary and Toxicity Report on o-Chlorobenzaldehyde with Attachments*, 22 October 1991. National Technical Information Service no. OTS0533437.

9. *Survey of Compounds for Radioactive Protection*, 1968. National Technical Information Service no. AD277689.

10. V. Plzak, J. Doull, *A Further Survey of Compounds for Radioactive Protection*, February 1969. National Technical Information Service no. AD691490.

11. W.P. Heilman, R.D. Battershell, W.J. Pyne, P.H. Goble, T.A. Magee, *Journal of Medicinal Chemistry*, 21 (1978) 906-913.

12. *The Merck Index*. 10th ed. 1983. Merck Co., Inc., Rathway, New Jersey. 1983.

13. H.F. Smyth, Jr., C.P. Carpenter, C.S. Weil, *Archives of Industrial Hygiene and Occupational Medicine*, 4 (1951) 119-122.

14. K. Nakamura, H. Ohyanagi, A. Suzuki, *Kobe Journal of the Medical Sciences*, 11 (1965) 63-71.

15. Environmental Protection Agency, U.S., Unpublished Test Data Report, *Initial Submission-1,2-Benzenedicarbonitrile-Acute Oral Toxicity Test in Rats and Skin Irritation Test in Guinea Pigs with Cover Letter Dated 073092*, 4 November 1980. National Technical Information Service no. OTS0540933.

16. National Defense Research Committee, Office of Scientific Research and Development, Progress Report, 132 (12/1942).

17. D.G. Upshall, *Toxicology and Applied Pharmacology*, 24 (1973) 45-59.

18. B. Ballantyne, D.W. Swanson, *Archives of Toxicology*, 40 (1978) 75-95.

19. J.R. Gaskins, R.M. Hehir, D.F. McCaulley, E.W. Ligon, Jr., *Archives of Environmental Health*, 24 (1972) 449-454.

20. S.M. Somani, (Editor), *Chemical Warfare Agents*, Academic Press, San Diego, 1992, p.271-288.

21. M. Nagarkatti, P.S. Nagarkatti, C.D. Raghuveeran, *Toxicology Letters*, 8 (1981) 73-76.

22. L. Leadbeater, G.L. Sainsbury, D. Utley, *Toxicology and Applied Pharmacology*, 25 (1973) 111-116.

23. Cucinelli, S.A., R.K. Biskup, S.C. Loure, H.L. Snodbrass, *Biochemical Toxicity of CA, CS, CN*, 1968. National Technical Information Service no. AD837-111.

24. C.L. Punte, J.T. Weimer, T.A. Ballard, J.L. Wilding, *Toxicology and Applied Pharmacology*, 4 (1962) 656-662.

25. K.I. Darmer, Jr., E.R. Kinkead, L.C. DiPasquale, *American Industrial Hygiene Association Journal*, 35 (1974) 623-631.

26. B.P. McNamara, *Edgewood Arsenal Technical Report No. EB-TR-76023.* U.S. Department of the Army (1976).

27. J.M. Harrison, T.D. Inch, *Tetrahedron Letters*, 22 (1985) 679-692.

28. *Material Safety Data Sheet: Pyrotechnic CS Agent.* Defense Technology Corporation of America (subsidiary of Armor Holdings GSA) (2/15/99).

**Table 4-1. CS-derived compounds observed at temperatures of 300-900 degrees Celsius**

| No. | Compound | Ret. Time (minutes) | m/z | Temperature | | | |
|---|---|---|---|---|---|---|---|
| | | | | 300 | 500 | 700 | 900 |
| 1 | 2-chlorobenzaldehyde | 8.05 | 139, 140 | X[2] | X | X | |
| | | | | X[2] | X | X | |
| | | | | X[c] | X | X[c] | |
| 2 | 2-chlorobenzonitrile | 8.22 | 137, 102 | | | X | X |
| | | | | | | X | X |
| | | | | | | X | X |
| 3 | quinoline | 9.82 | 129, 102 | | | | X[1] |
| | | | | | | | X[1] |
| | | | | | | | X[1] |
| 4 | 2-chlorobenzylcyanide | 10.50 | 116, 151 | | | | |
| | | | | | | | |
| | | | | | | | |
| 5 | 1,2-dicyanobenzene | 11.01 | 128, 101 | | | X[2] | X |
| | | | | | | X | X |
| | | | | | | | X |
| 6 | 3-(2-chlorophenyl)propynenitrile | 11.52 | 161, 163 | | | X | X[2] |
| | | | | | | X | X[2] |
| | | | | | | X | X |
| 7 | *cis* 2-chlorocinnamonitrile | 11.77 | 128, 163 | | | X | |
| | | | | | | X[2] | |
| | | | | | | X | |
| 8 | 2, 2-dicyano 3-(2-chlorophenyl) oxirane | 11.94 | 141, 89 | | | | X[1] |
| | | | | | | | X[1] |
| 9 | 2-chlorohydrocinnamonitrile | 11.97 | 125, 127 | | | | |
| | | | | | | | |
| | | | | | | | |
| 10 | benzylidenemalononitrile | 12.69 | 154, 127 | | | X | X[2] |
| | | | | | | X | X[2] |
| | | | | | | X | X[c] |
| 11 | *trans* 2-chlorocinnamonitrile | 12.80 | 128, 163 | | | X | X[2] |
| | | | | | | X | |
| | | | | | | X | X[1] |
| 12 | *cis* 2-cyanocinnamonitrile | 13.00 | 127, 154 | | | X[1] | X[1] |
| | | | | | | X[1] | X[1] |
| | | | | | | X[1] | X[1] |
| 13 | 2-chlorobenzylmalononitrile | 13.36 | 125, 127 | X | X | X | |
| | | | | X | X | X | |
| | | | | X | X | X | |
| 14 | 3-quinoline carbonitrile | 13.36 | 154, 127 | | | X[2] | X |
| | | | | | | X[2] | X |
| | | | | | | X[c] | X |
| 15 | 2-chlorobenzylidenemalononitrile | 13.90 | 153, 188 | X | X | X | X |
| | | | | X | X | X | X |
| | | | | X | X | X | X |
| 16 | *trans* 2-cyanocinnamonitrile | 14.39 | 127, 154 | | | | |
| | | | | | | | |
| | | | | | | | |
| 17 | 3-isoquinoline carbonitrile | 14.82 | 154, 127 | | | X | X |
| | | | | | | X | X[1] |
| | | | | | | X | X[c] |
| 18 | 4-chlorobenzylidenemalononitrile | 14.90 | 153, 188 | X[2] | X[2] | X[2] | |
| | | | | X[2] | X[2] | X[2] | |
| | | | | X[c] | X[c] | X[c] | |

X chromatogram showed presence of a peak greater than three times the background noise.
X[1] extracted ion chromatogram showed presence of base peak.
X[c] extracted ion chromatogram showed presence of the base peak and second largest m/z present.

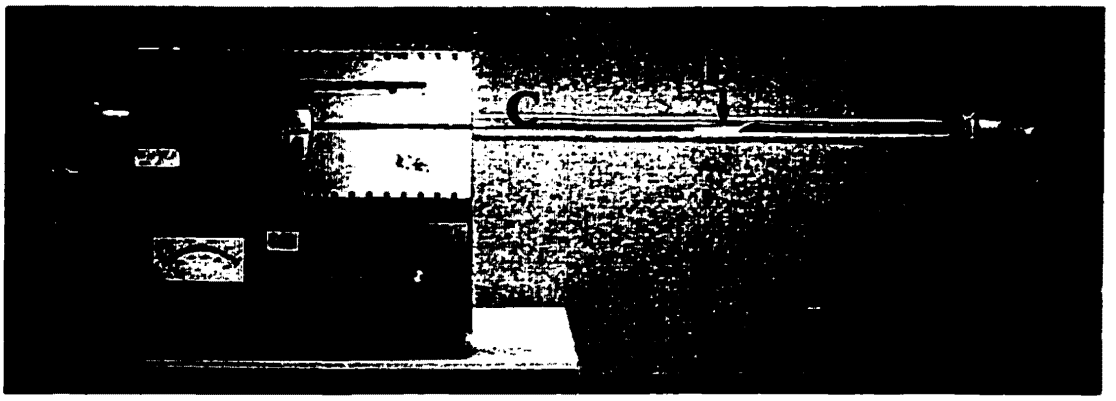


**Figure 4-1.** Front view of the system used to thermally degrade CS Riot Control Agent in a tube furnace and capture the resulting analytes.

(a) impinger

(b) tube furnace

(c) quartz tube

(d) combustion boat

(e) nitrogen line

(f) septum

(g) pushrod

# CHAPTER 5

# CONCLUSION

## *DISCUSSION OF RESEARCH FINDINGS*

In Chapter 2, it was shown that semi-volatile organic air contaminants produced by thermal dispersion of CS from a canister in a closed space were captured on a polytetraflouroethylene filter, desorbed using dichloromethane, separated from each other by open tubular gas chromatography, and detected using mass spectrometry. Compounds observed in addition to CS and its isomer 4-chlorobenzylidenemalononitrile included 2-chlorobenzaldehyde, 2-chlorobenzonitrile, quinoline, 2-chlorobenzylcyanide, 1,2-dicyanobenzene, 3-(2-chlorophenyl)propynenitrile, *cis* and *trans* isomers of 2-chlorocinnamonitrile, 2,2-dicyano-3-(2-chlorophenyl)oxirane, 2-chlorodihydrocinnamonitrile, benzylidenemalononitrile, *cis* and *trans* isomers of 2-cyanocinnamonitrile, 2-chlorobenzylmalononitrile, 3-quinoline carbonitrile, and 3-isoquinoline carbonitrile. These findings indicate that the CS-derived compounds observed were likely produced through rearrangements and by loss of cyano and chlorine substituents present on the parent CS compound.

Chapter 3 detailed sampling under similar conditions, and analysis for inorganic salts using the National Institute of Occupational Safety and Health methods 7904 and 6010 (modified) for HCN, and 7903 for HCl. The study showed that HCN was present in air samples collected during high temperature dispersion of CS. The presence of chloride in air samples suggests that HCl was present as well. HCN was found to be present in samples at levels in excess of the exposure level guidelines recommended by ACGIH and

NIOSH from the dispersion of two CS canisters inside a 240 $m^3$ riot control agent training chamber.

It was hypothesized that the formation of potentially harmful CS-derived compounds produced through free radical intermediates (*cis* and *trans* isomers of 2-cyanocinnamonitrile, 3-quinoline carbonitrile, and 3-isoquinoline carbonitrile), and the release of HCN (evidenced by the presence of 3-(2-chlorophenyl)propynenitrile), is temperature dependent. This led to Chapter 4, a third study where CS was heated in an inert atmosphere using a tube furnace. Pure CS was used so that the effect of temperature on CS could be analyzed independent of the other compounds present in canisters such as potassium chlorate, sugar, magnesium carbonate, and nitrocellulose. It was assumed this effect on the production of CS-derived compounds could be generalized to that formed by high-temperature dispersion of CS canisters. By assuming that neat CS behaves in a similar manner as that found in canisters when exposed to heat, standardizing residence time in the tube furnace, and using an inert nitrogen carrier gas at a constant flow, it was shown that many of the organic degradation products observed earlier are produced through heating.

Caution must be taken when attempting to generalize conclusions drawn from this laboratory-based CS data to exposures that occur during thermal dispersion of CS in the field. However, it is necessary to conduct laboratory experiments that control for variables associated with dispersal such as temperature, wind speed, humidity, and other compounds present in the dispersal process. This permits isolation of the effect of a single variable on CS alone as shown in the third study involving temperature.

*PUBLIC HEALTH RELEVANCE*

*Overview.* The public health issues attributed to acute CS exposure have been thoroughly studied as discussed in the introduction. However, the research described here has shown that CS may degrade to produce potentially harmful compounds when it is thermally dispersed. Most of these CS-derived compounds have not been evaluated for potential to produce acute or chronic effects. Acute toxicity data for CS and CS-derived compounds can be found in Table 5-1. Additionally, the current NIOSH-recommended methods for analysis of CS and CS-derived compounds are less than adequate given the current arsenal of instrumental and analytical techniques now available to analysts. We must ask ourselves, is it safe to surmise that further research will alleviate public health concerns surrounding exposure to CS and CS-derived compounds by vindicating its use, or will it complicate matters by implicating new variables and endpoints that have gone unstudied? The process should begin with a comprehensive assessment of human health risks.

*Health Risk Assessment.* As mentioned previously, the identification of the compounds of concern, followed by toxicity assessment, exposure assessment, and risk characterization are the four steps of a health risk assessment designed to estimate the increased cancer and non-cancer hazard risks posed due to exposure to the compounds of concern. The manuscripts presented earlier titled "*Identification of CS-derived Compounds formed during Heat-dispersion of CS Riot Control Agent;*" "*Liberation of Hydrogen Cyanide and Hydrogen Chloride during High Temperature Dispersion of CS Riot Control Agent;*" and "*Formation of 2-chlorobenzylidenemalononitrile (CS Riot Control Agent) Thermal Degradation Products at Elevated Temperature*" may be

considered collectively as part of the initial step of a comprehensive HRA of CS and CS-derived compounds produced by heat-dispersion.

Secondly, toxicological dose-response studies should be obtained for each CS-derived compound to which human exposure may occur. Acute animal toxicity studies (Table 5-1) evaluating the dose response relationships of CS and CS-derived compounds exist for only 8 of the 20 compounds [43-60] hence, at least 12 additional dose-response studies need to be performed assuming that the previously cited studies were satisfactorily completed. Additionally, most of the compounds that have had toxicological studies performed to identify lethal dose and lethal concentration endpoints, evaluated intraperitoneal, oral, and injection exposure pathways, excluding either the dermal or inhalation exposure routes that are consistent with traditional CS exposure.

Thirdly, a collective exposure assessment identifying exposure groups, exposure routes (dermal, inhalation, ingestion), contaminant concentrations, exposure duration, and contaminant intake values for each pathway must be produced. It would be quite a task to attempt to quantify the number of individuals who are exposed to thermally dispersed CS on an annual basis.

Lastly, the process concludes with a risk characterization for exposure to the compounds of concern, estimating the overall increase of cancer and non-cancer hazard risk due to such exposure.

*RECOMMENDATIONS FOR FUTURE RESEARCH*

Further research is warranted to fulfill the remaining health risk assessment process requirements necessary to assess cancer and non-cancer health risks associated

with exposure to the compounds we have identified. Previous assessments of human CS exposure have focused on CS alone, not accounting for the CS-derived thermal degradation products [6, 19, 28-32]. A more comprehensive health risk assessment requires the identification of all compounds of concern to which people may be exposed in addition to the parent compound or starting material.

The study addressing the effect of temperature on CS-derived compound formation concluded that 3-(2-chlorophenyl)propynenitrile among other compounds, was produced when CS was heated to temperatures greater than 700°C in the system used. HCN levels may increase as well because HCN is very likely formed as a result of the loss of HCN from the CS molecule in the formation of 3-(2-chlorophenyl)propynenitrile. This should be tested in the laboratory by placing the appropriate sorbent material in series with a tube furnace apparatus to capture and quantify the amount of HCN produced.

Current military mask confidence and readiness training commonly occurs in a chamber or tent and involves heating a capsulated powder form of CS to produce a vapor that condenses to an aerosol. Further investigation is needed to rule out the possibility that this method produces the CS-derived compounds observed in our study. If CS-derived compounds are observed during heating of capsulated powder, a study should be designed to evaluate the chemical protective gear's ability to minimize exposure to them.

The military sometimes performs combat training outdoors using CS canisters and smoke obscurants simultaneously. Training of this type should be evaluated for possible synergistic effects from exposure to the total mixture encountered.

Law enforcement and correctional officer training sometimes involves the use of CS canisters in enclosed trailers or similar training facilities. There have been instances where people have been exposed to canisters indoors during extraction procedures and rioting. Law enforcement and correctional officers often train with CN and oleoresin capsicum (OC) in addition to CS. Possible synergistic effects resulting from exposure to mixtures of these substances should be explored as well.

*CONCLUSION*

The research described in this dissertation has identified eighteen organic and two inorganic compounds to be present during heat-dispersion of canister CS. Also presented was a study describing the CS-derived compounds observed at temperatures of 300, 500, 700, and 900°C in a laboratory system. The findings of this research, performed in partial fulfillment of the requirements for the degree of Doctor of Public Health, support the conclusion that a re-assessment of human health risks associated with exposure to CS riot control agent dispersed at high temperature should be conducted, and should consider the full range of contaminants produced during the high-temperature dispersion process. Furthermore, it is recommended that canister manufacturers conduct research aimed at reducing the production of potentially harmful CS-derived compounds such as hydrogen cyanide so that exposure to these compounds is minimized.

**Table 5-1. Acute toxicity LD50 and LC50 values for CS and CS-derived thermal degradation products**

| COMPOUND | ANIMAL | ROUTE | LD50/LC50 | REFERENCE |
|---|---|---|---|---|
| 2-chlorobenzaldehyde | rat | oral | 2160 mg/kg | [43] |
| | mouse | intraperitoneal | 10 mg/kg | [44] |
| | mouse | oral | 1900 mg/kg | [43] |
| 2-chlorobenzonitrile | mouse | intraperitoneal | 150 mg/kg | [45] |
| | mouse | oral | >300 mg/kg | [46] |
| quinoline | rat | oral | 460 mg/kg | [47] |
| | rabbit | skin | 540 uL/kg | [48] |
| 2-chlorobenzylcyanide | * | * | * | |
| 1,2-dicyanobenzene | rat | intraperitoneal | 62 mg/kg | [49] |
| | rat | oral | 30 mg/kg | [50] |
| | mouse | intraperitoneal | 25 mg/kg | [44] |
| | guinea pig | skin | >1 gm/kg | [50] |
| 3-(2-chlorophenyl)propynenitrile | * | * | * | |
| cis 2-chlorocinnamonitrile | * | * | * | |
| 2, 2-dicyano 3-(2-chlorophenyl)oxirane | * | * | * | |
| 2-chlorohydrocinnamonitrile | * | * | * | |
| benzylidenemalononitrile | mouse | inhalation | 1450 mg/m$^3$/10M | [51] |
| trans 2-chlorocinnamonitrile | * | * | * | |
| cis 2-cyanocinnamonitrile | * | * | * | |
| 2-chlorobenzylmalononitrile | * | * | * | |
| 3-quinoline carbonitrile | * | * | * | |
| 2-chlorobenzylidenemalononitrile | rat | intraperitoneal | 48 mg/kg | [52] |
| | rat | intravenous | 28 mg/kg | [53] |
| | rat | oral | 178 mg/kg | [54] |
| | rat | inhalation | 88,480 mg/m$^3$/1M | [55] |
| | mouse | intraperitoneal | 32 mg/kg | [56] |
| | mouse | intravenous | 48 mg/kg | [23] |
| | mouse | oral | 282 mg/kg | [57] |
| | rabbit | intravenous | 8 mg/kg | [58] |
| | rabbit | oral | 143 mg/kg | [53] |
| | rabbit | inhalation | 50,000 mg/m$^3$/1M | [55] |
| | guinea pig | intraperitoneal | 73 mg/kg | [53] |
| | guinea pig | oral | 212 mg/kg | [53] |
| | guinea pig | inhalation | 54,100 mg/m$^3$/1M | [55] |
| trans 2-cyanocinnamonitrile | * | * | * | |
| 3-isoquinoline carbonitrile | * | * | * | |
| 4-chlorobenzylidenemalononitrile | * | * | * | |
| hydrogen chloride | rat | inhalation | 4701 ppm/30M | [59] |
| | mouse | inhalation | 2644 ppm/30M | [59] |
| hydrogen cyanide | human | inhalation | 524 ppm/10M | [60] |

* no information was found through the literature search conducted.

## BIBLIOGRAPHY


1. Crawley, R., *The history of the Peloponnesian War.* 1974. Avon Publishing, Avon, Connecticut.
2. Robinson, J.P., *Problem of chemical and biological warfare: a study of historical, technical, military, legal, and political aspects of CBW. Vol 1. The rise of CB weapons.* 1971. Humanities Press, New York, New York.
3. Barrow, R.H., *Plutarch and his times.* 1971. Indiana University Press, Bloomington, Indiana.
4. Gilchrist, H.L., *A comparative study of warfare gases, their history, description and medical aspects.* 1925. Medical Field Service School, Carlisle Barracks, Pennsylvania.
5. Hague Peace Conference, *Declaration II: Use of projectiles the object of which is the diffusion of asphyxiating or deleterious gases.* 29 July 1899. Netherlands.
6. Elskamp, D.M.W., *Toxic properties of CN and CS.* December 1976. Medisch Biologisch Laboratorium, Netherlands.
7. *Protocol for the prohibition of the use in war of asphyxiating, poisonous or other gases, and of bacteriological methods of warfare. Hague Convention.* 1925. Geneva, Switzerland.
8. *Executive Order 11850, Renunciation of certain uses in war of chemical herbicides and riot control agents.* 1976.
9. Sidell, F.R., in Zajtchuk, R. (Editor), *Textbook of military medicine, medical aspects of chemical and biological warfare.* 1997. Office of the Surgeon General

at TMM Publications, Borden Institute, Walter Reed Army medical Center, Washington, D.C.

10. Corson, B.B. and R.W. Stoughton, *Reactions of alpha, beta-unsaturated dinitriles.* Journal of the American Chemical Society, 1928. 50: p. 2825-2837.

11. Department of the Army, U.S., *Field Manual 3-11. Flame, riot control agents, and herbicide operations.* 19 August 1996.

12. Memorandum, *Treasury Department Press.* 13 July 1995.

13. Silva, D. and R. Lewis, *Tear gas debuts at WTO.* 30 November 1999. Environmental News Service, Seattle, Washington.

14. National Institute of Occupational Safety and Health (NIOSH): *P&CAM 304, o-chlorobenzylidenemalononitrile.* Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Issued April 1979.

15. Settle, F.A., *Handbook of instrumental techniques for analytical chemistry.* 1997. Prentice Hall PTR, Upper Saddle River, New Jersey.

16. Thomas, R.J., P.A. Smith, D.A. Rascona, J.D. Louthan, and B. Gumpert, *Acute pulmonary effects from CS "gas" (Ortho-chlorobenzylidene malononitrile) exposure in a US Military training exercise.* Military Medicine, 2000 (submitted).

17. Smith, P.A., T.A. Kluchinsky, Jr., P.B. Savage, R.P. Erikson, A.P. Lee, K. Williams, M. Stevens, and R.J. Thomas, *Traditional sampling with laboratory analysis and solid phase microextraction sampling with field gas chromatography/mass spectrometry by military industrial hygienists.* Journal of the American Industrial Hygiene Association, 2000 (submitted).

18. Department of the Army, U.S., *Process for making epoxides from ethylenic compounds with electron-withdrawing groups, United States Patent Office.* Patent Number 3,313,829. 1967.

19. Jones, G.R.N., *CS and its chemical relatives.* Nature, 1972. 235: p. 257-261.

20. Sax, N.I. and R.J. Lewis, Sr, *Dangerous properties of industrial materials.* 7th ed. Vol. III. 1989. Von Nostrand Reinhold, New York, New York.

21. Solomons, T.W.G., *Organic chemistry.* Fourth ed. 1988. Wiley & Sons, Inc., New York, New York.

22. *National Institute of Occupational Safety and Health (NIOSH), Pocket Guide to Chemical Hazards, 2000.* U.S.Government Printing Office, Washington, D.C.

23. Leadbeater, L., G.L. Sainsbury, and D. Utley, *ortho-chlorobenzylmalononitrile: A metabolite formed from ortho-chlorobenzylidenemalononitrile (CS).* Toxicology and Applied Pharmacology, 1973. 25: p. 111-116.

24. Department of the Army, U.S., *Field Manual 8-9. NATO Handbook on the medical aspects of NBC defensive operations.* 1 February 1996.

25. Owens, E.J. and C.L. Punte, *Human respiratory and ocular irritation studies utilizing o-chlorobenzylidene malononitrile aerosols.* American Industrial Hygiene Association Journal, 1963. 24: p. 262-264.

26. Department of the Army, U.S., *U.S. Army, Chemical Corps Safety Directive No. 385-12. Safety Guide for the processing, filling, handling and decontamination of CS and CS1.* 1961. Edgewood Arsenal, Maryland.

27. Department of the Army, U.S., *Technical Manual 3-215. Military chemistry and chemical agents.* 1 December 1963.

28. Punte, C.L., E.J. Owens, and P.J. Gutentag, *Exposure to ortho-chlorbenzylidene malononitrile.* Archives of Environmental Health, 1963. 6: p. 72-80.

29. Weigand, D., *Cutaneous reaction to the riot control agent CS.* Military Medicine, 1969. 134: p. 437-440.

30. Holland, P. and R.G. White, *The cutaneous reactions produced by ortho-chlorobenzylidenemalononitrile and alpha-chloroacetophenone when applied directly to the skin of human subjects.* British Journal of Dermatology, 1972. 86: p. 150-154.

31. Beswick, F.W., P. Holland, and K.H. Kemp, *Acute effects of exposure to orthochlorobenylidene malononitrile (CS) and the development of tolerance.* British Journal of Industrial Medicine, 1972. 29: p. 298-306.

32. Cotes, J.E., J.M. Dabbs, M.R. Evans, and P. Holland, *Effects of CS aerosol upon lung gas transfer and alveolar volume in healthy men.* Quarterly Journal of Experimental Physiology, 1972. 57:199-206.

33. Wils, E.R.J. and A.G. Hulst, *Mass spectra of some derivatives of the irritant o-chlorobenzylidenemalononitrile (CS).* Fresenius Zeitschrift fur Analytische Chemie. 1985. 320: p. 357-360.

34. Wils, E.R.J. and A.G. Hulst, *Gas chromatographic-mass spectrometric identification of tear-gases in dilute solutions using large injection volumes.* Journal of Chromatography, 1985. 330: p. 379-382.

35. Harrison, J.M. and T.D. Inch, *A novel rearrangement of the adduct from CS-epoxide and dioxan-2-hydroperoxide.* Tetrahedron Letters, 1985. 22: p. 679-692.

36. Grob, R.L., *Modern practice of gas chromatography*. Third ed. 1995. Wiley & Sons, Inc., New York, New York.

37. Sass, S., T.L. Fisher, M.J. Jascot, and J. Herban, *Gas-liquid chromatography of some irritants at various concentrations*. Analytical Chemistry, 1971. 43: p. 462-464.

38. Ferslew, K., E, R.H. Orcutt, and A.N. Hagardorn, *Spectral differentiation and gas chromatographic/mass spectrometric analysis of the lachrymators 2-chloroacetopheone and ortho-chlorobenzylidene malononitrile*. Journal of Forensic Sciences, 1986. 31, No. 2, April 1986: p. 658-665.

39. Martz, R.M., D.J. Reutter, and L.D.I. Lasswell, *A comparison of ionization techniques for gas chromatography/mass spectrometry analysis of dye and lachrymator residues from exploding bank security devices*. Journal of Forensic Sciences, 1983. 28(No. 1): p. 200-207.

40. Nowicki, J., *Analysis of chemical protection sprays by gas chromatography / mass spectroscopy*. Journal of Forensic Science, 1981. 27(No. 3): p. 704-709.

41. Tripathi, D.N., R.C. Malhorta, and A. Bhattacharya, *Gas chromatographic-mass spectrometric identification of alpha-chloroacetophenone, ortho-chlorobenzylidene malononitrile and dibenz[b,f]-1:4-oxazepine*. Journal of Chromatography, 1984. 315: p. 417-420.

42. Environmental Protection Agency, U.S., *Risk Assessment Guidance for Superfund, Volume I, Human Health Evaluation Manual (Part A)*. 1989.

43. Environmental Protection Agency, U.S., Unpublished Test Data Report, *Letter from Eastman Kodak Company to USEPA Submitting Enclosed Toxicity and*

*Health Summary and Toxicity Report on o-Chlorobenzaldehyde with Attachments.* 22 October 1991. National Technical Information Service no. OTS0533437.

44. *Survey of Compounds for Radioactive Protection,* 1968. National Technical Information Service no. AD277689.

45. Plzak, V., and J. Doull, *A Further Survey of Compounds for Radioactive Protection.* February 1969. National Technical Information Service no. AD691490.

46. Heilman, W.P., R.D. Battershell, W.J. Pyne, P.H. Goble, and T.A. Magee, *Synthesis and antiinflammatory evaluation of substituted isophthalnoitriles, trimesonitriles, benzonitriles, and terephthalonitriles.* Journal of Medicinal Chemistry, 1978. 21: p. 906-913.

47. The Merck Index. 10th ed. 1983. Merck Co., Inc., Rathway, New Jersey. 1983.

48. Smyth H.F., Jr., C.P. Carpenter, and C.S. Weil, *Range-finding toxicity data: list IV.* Archives of Industrial Hygiene and Occupational Medicine, 1951. 4: p. 119-122.

49. Nakamura, K., H. Ohyanagi, and A. Suzuki, *Histological studies on the rat brain in case of acute pthalodinitrile intoxication.* Kobe Journal of the Medical Sciences, 1965. 11: p. 63-71.

50. Environmental Protection Agency, U.S., Unpublished Test Data Report, *Initial Submission-1,2-Benzenedicarbonitrile-Acute Oral Toxicity Test in Rats and Skin Irritation Test in Guinea Pigs with Cover Letter Dated 073092.* 4 November 1980. National Technical Information Service no. OTS0540933.

51. National Defense Research Committee, Office of Scientific Research and Development, *Progress Report, 132* (12/1942).

52. Upshall, D.G., *Effects of o-chlorobenzylidene malononitrile (CS) and the stress of aerosol inhalation upon rat and rabbit embryonic development.* Toxicology and Applied Pharmacology, 1973. 24: p. 45-59.

53. Ballantyne, B., and D.W. Swanson, *The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS).* Archives of Toxicology, 1978. 40: p. 75-95.

54. Gaskins, J.R., R.M. Hehir, D.F. McCaulley, and E.W. Ligon, Jr., *Lacrimating agents (CS and CN) in rats and rabbits.* Archives of Environmental Health, 1972. 24: p. 449-454.

55. Somani, S.M., *Chemical warfare agents.* 1992. Academic Press, Inc., San Diego, California.

56. Nagarkatti, M., P.S. Nagarkatti, and C.D. Raghuveeran, *Short-term toxicity studies of o-chlorobenzylidene malononitrile on humoral immunity in mice.* Toxicology Letters, 1981. 8: p. 73-76.

57. Cucinelli, S.A., R.K. Biskup, S.C. Loure, and H.L. Snodbrass, *Biochemical Toxicity of CA, CS, CN.* 1968. National Technical Information Service no. AD837111.

58. Punte, C.L., J.T. Weimer, T.A. Ballard, and J.L. Wilding, *Toxicologic studies on o-chlorobenzylidene malononitrile.* Toxicology and Applied Pharmacology, 1962. 4: p. 656-662.

59. Darmer, K.I., Jr., E.R. Kinkead, and L.C. DiPasquale, *Acute toxicity of rats and mice exposed to hydrogen chloride gas and aerosols.* American Industrial Hygiene Association Journal, 1974. 35: p. 623-631.

60. McNamara, B.P., *Estimation of the toxicity of hydrocyanic acid vapors in man.* 1976. Edgewood Arsenal Technical Report no. EB-TR-76023.